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Section B.—Biological Sciences.

The Influence of Osmotic Pressure upon the Regeneration of Gunda ulvæ.

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(Communicated by Prof J. Stanley Gardiner, F R S. Received March 21,—Read April 30, 1914)

CONTENTS.

I Introduction.

This work was carried out during the summer of 1912 and the spring of 1913, at the laboratory of the Marine Biological Association, at Plymouth. The object of the investigation was to discover how far marine turbellarians could be made to undergo variation while regenerating lost parts, and, if possible, to correlate the variations with definite physical conditions. *Gunda ulvæ* was the only species used during the course of the investigation It is a small Triclad and its structure has been described in detail by Wendt (16) and Ijima (5).

The length of the fully grown specimens obtained at Plymouth lies between 5 and 6 mm., though the worms are found sexually mature from a length of 4 mm It occurs between tide-marks and near the borders of a small stream Density determinations made on different occasions of the water in which it was found, show that it is normally exposed to a very wide variation in osmotic pressure The lowest density value recorded was 1 001. *i e* a value corresponding to almost fresh water, the highest was 1 028 The mean density of the sea-water at Plymouth is 1·023 This fact is an important one to take into consideration when placing the animal under experimental conditions in the laboratory.

Before proceeding to the main part of the work, it seemed advisable to make three sets of preliminary experiments· the first of these was to determine the limits of osmotic pressure within which whole worms are capable of living; the second was to observe the morphological changes accompanying starvation in whole animals, and the third was to make a histological study of the regeneration in sea-water These are all described in detail below

For the experiments in regeneration, only regeneration of the posterior end was considered *G ulvæ* differs from most Triclads in that it will only regenerate a new head in the presence of the cerebral ganglia (6) As it was wished to study the effects of removing large pieces, the experiments were necessarily limited to regeneration of the posterior end To secure uniformity, the worms were all transected half way down the length of the body

G ulvæ is capable of living for many months in a state of starvation, during which it slowly diminishes in size Nevertheless, worms kept without food until reduced in length from 5 5 to 1 5 mm, when bisected, restored their normal form as rapidly as did fully fed specimens This point is emphasised here because in the experiments considered in this paper the animals were left entirely without food This was done, firstly, to avoid contaminating the experimental waters, and, secondly, because worms regenerating in different solutions develop the new mouth at very different intervals after section, and thus the starvation experiments progress equally rapidly and more uniformly A number of observations were made on the regeneration of *G ulvæ* under both starving and fully fed conditions, and the differences which are found consist only in the rather less degree of reduction that occurs when feeding is taking place while the worms regenerate.

The figures of the whole worms are camera sketches made from living material The sections were made from material fixed in corrosive acetic, and stained in picronigrosin and eosin. The sections used for studying the

cytology were stained with Heidenhain's iron hæmatoxylin followed by orange G

II Preliminary Experiments on Reduction and Growth in Whole Animals

(a) *Reduction*

A number of adult worms 5–6 mm in length were washed in sterile water and placed in a wide-mouthed sterile bottle which was closed with a cork Through this cork were passed two glass tubes forming the inlet and outlet of an air-circulation, both tubes having a cotton-wool plug in them to keep back chance organisms in the circulating air. The animals in this apparatus were kept in a state of starvation At intervals animals were removed, measured, and fixed for sectioning

The worms were found to decrease fairly steadily in length under starvation Curve A in text-fig 1 shows the course of diminution in length in an experiment lasting 12 weeks Microscopical examination of sections showed that

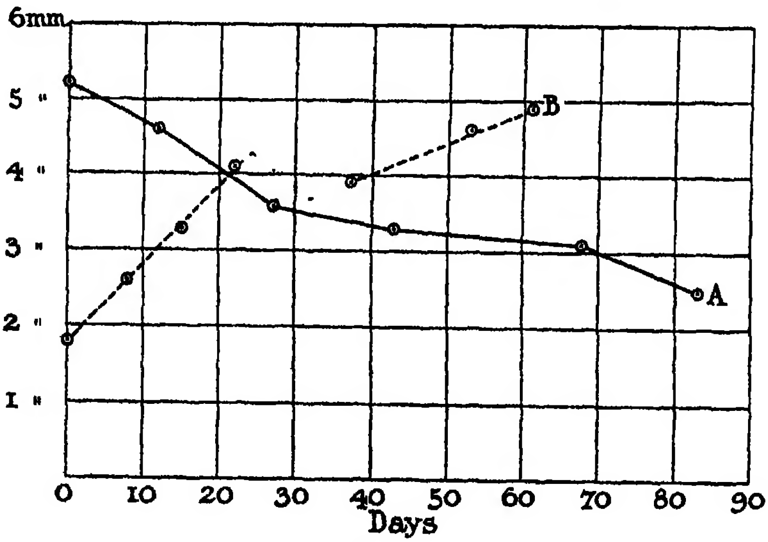


Fig 1

Curve A, curve of diminution of length of starving animals. Curve B, curve of rate of growth of starved worms on feeding Note that feeding was interrupted between 22nd and 37th day.

starvation is always accompanied by absorption of the generative system. The yolk-glands appear to be absorbed first, but after the worms have been reduced to about 4–5 mm in length degenerative changes appear in the ovary, testes and secondary sexual organs. By the time the worms are reduced

to 3 mm in length, *i.e* after 10 weeks, the last trace of the generative system has vanished.

Stoppenbrink (15) has recorded similar occurrences in starving *Planaria*. He states that the order of disappearance is.—Yolk-glands, copulatory apparatus, testes, and ovaries. In *Gunda*, while the yolk-glands disappear first, the rest of the genital system appears to be absorbed at a uniform rate. Stoppenbrink states that there is no phagocytosis in the absorption of the organs In *Gunda* the fact that an organ undergoing reduction is always surrounded by a sheath of parenchyma cells (see fig 4, B), some of which even penetrate into the various organs, makes it appear probable that the organs are destroyed by a phagocytic action of the undifferentiated parenchyma. Fig 2 shows an adult testis, fig 3 a testis undergoing degeneration Figs 4, A and B, show the changes in the ovary

Fig 2 —Testis of Adult Worm.
g, gut *tes*, testis. *y gl*, yolk-glands

Fig. 3.—Testis after 21 Days' Regeneration of Posterior End in Sea-water.
deg.sp.c., degenerating spermatocytes. *tes.*, testis.

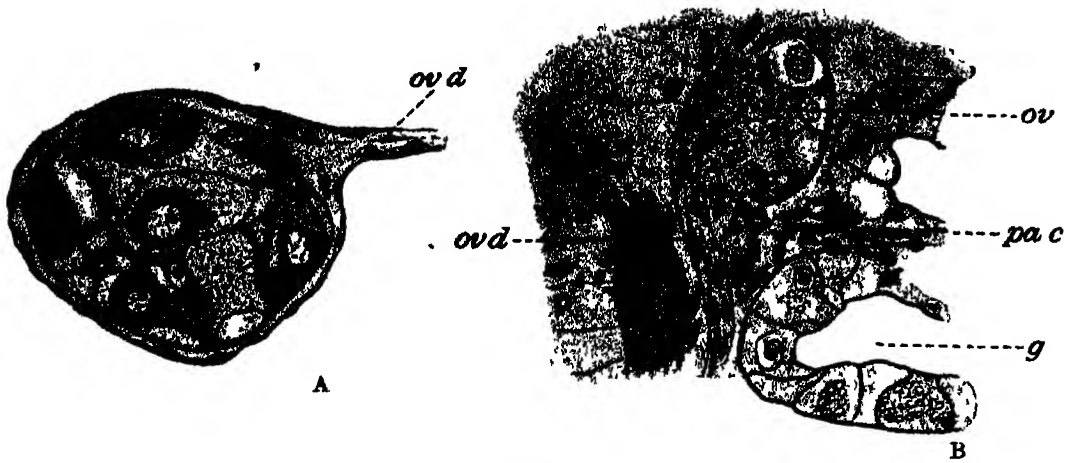


FIG 4

A —Ovary

B —Ovary after 25 days' regeneration of posterior end in sea-water

g, gut *pa c*, parenchyma cells. *ov*, ovary *ov d*, oviduct

(b) *Growth.*

Experiments were also made in rate of growth Worms which had been reduced to 1 5 mm in length by a long period of starvation were fed daily on scraps of the tail muscle of shrimps The rate of growth was very rapid, and is indicated in curve B, in fig 1 Unfortunately the experiment was interrupted, and the worms went without food from the 25th to the 37th day It can be seen that this period was one of reduction in length On the feeding being resumed growth again took place

At the beginning of the experiment the worms were without a trace of the generative system This was first noticed in the sections at the 10th day, *i e* when the worms were 2 7 mm long The generative system was completely restored at a length of 4 6 mm, *i e* 36 days after its first appearance The generative system in *Gunda*, as in other Turbellaria, is formed by the localisation and metamorphosis of nests of parenchyma cells

III Preliminary Experiments on Duration of Life of Whole Animals in Solutions of Different Osmotic Pressure

A preliminary series of experiments was made so as to ascertain the limits of density, salinity, and osmotic pressure which whole worms are capable of withstanding A table recording these results is given below (Table II) Conditions under which the worms can live for seven weeks were taken as capable of supporting them alive indefinitely.

Table I

	Composition of water	Density	Salinity	Osmotic pressure (atmospheres) (approx within 0 5 atmosphere)	Length of life of *G ulvæ* in the solution.
			grm per litre		
1	Distilled water	1 ·000	0 00	—	1–18 hours
2	Tap water	1 ·000	0 00	—	6 hours–3 days
3	100 c c distilled water— + 5 c c sea water	1 ·000	1 60	1 0	3–6 days
4	+ 10 " "	1 001	3 11	2 0	Indefinitely
5	+ 20 " "	1 002	5 70	3 5	"
6	+ 50 " "	1 ·007	11 4	7 5	"
7	+ 100 " "	1 012	17 1	11 ·0	"
8	+ 200 " "	1 ·015	22 ·8	15 0	"
9	+ 500 " "	—	28 5	18 5	"
10	Sea-water	1 023	34 2	22 5	"
11	100 c c sea-water— + 10 c c 2 5M NaCl	1 030	44 3	29 5	"
12	+ 20 " "	1 ·037	52 8	33 5	14 days–Indefinitely
13	+ 30 " "	1 043	60 1	40 5	18–40 days
14	+ 40 " "	1 046	66 7	—	5–20 "
15	+ 80 " "	1 058	83 8	-	3–7 "
16	+ 200 " "	1 ·073	109 0	—	6 hours–1 day

The solutions used were made by mixing known volumes of sea-water with distilled water, or with 2 5M NaCl, calculating the salt content, and from this taking the osmotic pressure from the values given in Krümmel's 'Handbuch der Ozeanographie' (8)

The salinity of the sea-water used was measured by titrating against standard silver nitrate and determining the chlorine value. Knudsen's tables (7) give the formula for converting the chlorine value obtained into the salinity value

It will be seen that the worms are capable of living for an indefinite time in water which has an osmotic pressure of more than 2 and less than 33 atmospheres Between these limits the worms remain perfectly normal in appearance In solutions more dilute and in fresh and distilled water the worms swell up to four or five times their normal size In water having a salinity of 66 per cent. or more they become shrivelled.

IV Normal Regeneration of the Posterior End

The changes which set in in *G. ulvæ* after section and which culminate in the restoration of the lost parts and production of a complete worm fall under the head of regulatory rather than of regenerative changes. There is no growth in the ordinary sense of the word, since the first product of

regulation is never larger than the fragment which produced it. Fig 5, 1–4, show the stages in the transformation of an anterior end into a complete worm It can be seen that there is in this case actual diminution in length This is due to the fact that the regulation occurred under starving conditions When the pieces are fully fed there is no reduction in length.

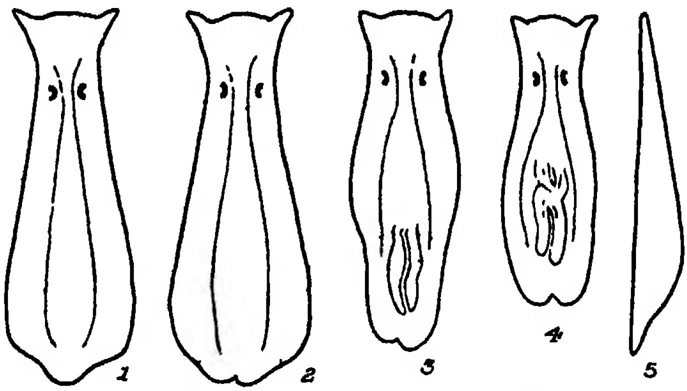


FIG 5 —Regeneration of Posterior End in Sea-water
1 7 days 2 14 days. 3 28 days 4 49 days. 5 Side view of 3

The chief *rôle* in the transformation is played by the parenchyma cells. These cells are small migratory cells which are found in large numbers throughout the whole length of the animal's body In regulation they have two functions—

(1) They migrate in large numbers to the region of the wound, where they form first an undifferentiated mass of cells and later the new tissues

(2) They act as phagocytes, making a sheath round the old organs, *e g*, brain and genitalia, and reducing them in size until they have become proportionate to the size of the new worm.

The course of regulation in *G ulvæ* is essentially similar to that already described in various species of *Planaria* by Schultz (11), Flexner (4), and Bardeen (1) The wound is closed by the ectoderm creeping inwards as a thin acellular layer (see fig 6, *ac ep*).

After the wound has healed (three to five days after section) the parenchyma accumulates at the hind end. This accumulation of parenchyma cells is chiefly due to migration Mitotic figures are to be found, but are not common in the sections, showing that actual cell division plays only a small *rôle* in the building up of the new tail. This consists for a few days of undifferentiated parenchyma, but later differentiated cells appear. The formation of the muscular layer of the new tail takes place by differentiation

Fig 6.—Regeneration of the posterior end of *G ulvæ* in sea-water 3 days. *ac ep*, acellular extension of epidermis *ep*, epidermis *m*, muscle *pa c*, parenchyma cell *ph*, pharynx *ph.c*, pharynx chamber *w*, point of closure of wound

Fig. 7.—Regeneration of the posterior end of *G ulvæ* in sea-water 14 days. *m.*, mouth *m.c.*, muscle cell. *pa.c*, parenchyma cell *ph.*, pharynx. *ph.c.*, pharynx chamber. *w.*, point of closure of wound.

of parenchyma cells, one parenchyma cell forming one differentiated muscle fibre (fig 7). The restoration of the circle of the nerve cords also takes place by parenchyma cells pushing their way into the cut end of the old nerve cords and becoming transformed into nerve cells. The new gut is formed by the cut ends of the two branches of the old gut fusing together behind the pharynx The wound on the pharynx is also healed by the migration of parenchyma cells

The new mouth is formed by a perforation appearing between the pharynx chamber and the exterior The mouth perforates at a point just anterior to the point of closure of the wound, usually about nine days after section (fig 7, *m* and *w*).

While the constructive changes described above have been taking place in the region of the wound, in the rest of the animal's body reductive change has been simultaneously proceeding The first system to show degenerative change is the generative system The amount of degeneration that takes place is directly proportional to the amount of restoration that is required In the present case, where regeneration is being considered after a cut that has removed half the body, reduction proceeds so far that the whole of the generative system is absorbed The yolk glands are absorbed first, then the genital glands and secondary sexual organs

Besides the absorption of the genital system it can be seen on referring to figs 5, 1–4, and 8, 1–4, that the growth in size of the posterior end is accompanied by a reduction in size of the anterior end This external adjustment is found to apply also to all the internal organs of the animal, *i.e.*, as the new parts grow larger the old grow smaller until the proper equilibrium is reached.

When these changes have restored the normal proportions of the worm, regulation is complete. The subsequent increase in size, and in the case under consideration, restoration of the generative system, are phenomena of normal growth (see Section II, *b*)

It might here be noticed that the reductional changes described above in a regenerating worm fragment are entirely identical with those described in Part II (*a*) of this paper, where the reduction was the result of starving whole animals. It will be seen later that, when regeneration is inhibited, reduction is inhibited to the same degree Absorption of the generative system takes 10 weeks in starving worms; in animals which have been bisected and are regenerating under starving conditions it takes 5–6 weeks In animals which have been bisected and are being fed while regenerating, the genital system is greatly reduced, but never completely absorbed

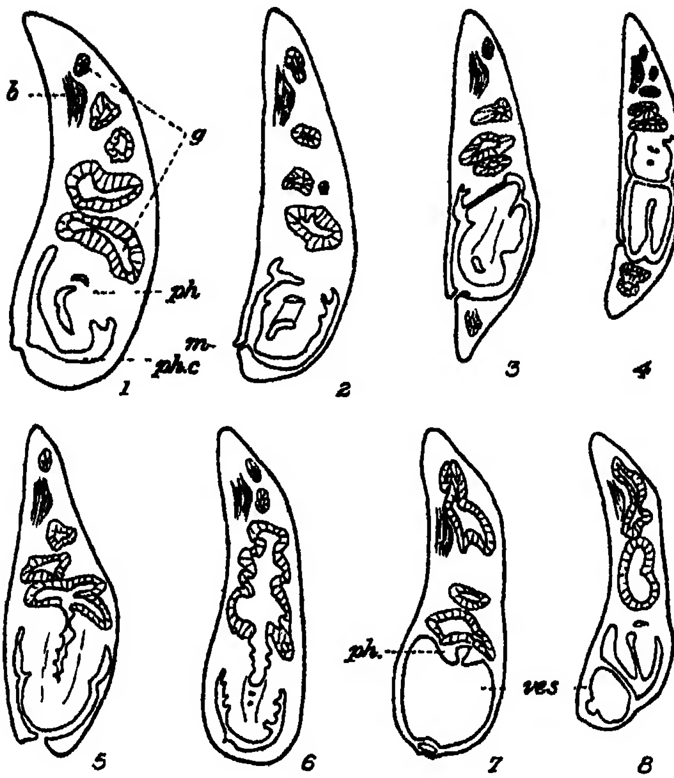


FIG 8

Median longitudinal sections through worm regenerating in sea-water—1 7 days 2 14 days. 3 21 days. 4 56 days.
Median longitudinal sections through worm regenerating in 100 c.c. sea-water + 20 c.c $2\frac{1}{2}$M NaCl.—5 7 days. 6 14 days 7 21 days. 8 31 days

V Regeneration under Varying Conditions of Osmotic Pressure.

A number of experiments in regeneration were set up in artificial solutions 4–11 of Table II, in order to see what effect was produced by raising or lowering the osmotic pressure of the medium It can be seen that a range of pressure of from 2 to 40 atmospheres was examined. The results of such experiments show that moving the osmotic pressure in either direction from a definite optimum value just below that of sea-water results in a decrease in the rate of regeneration. This decrease of rate culminates, at 2 and 40 atmospheres respectively, in complete inhibition of the restorative processes Fig. 9 shows a number of worms on the 42nd day of regeneration, which have been taken from different experimental media.

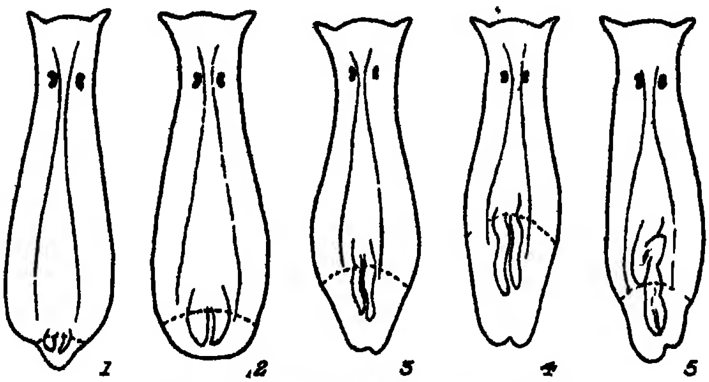


FIG 9 — Worms 42 days after section from—

1. 100 c c distilled water + 50 c c sea-water 2 100 c.c. distilled water + 100 c.c. sea-water. 3 100 c.c. distilled water + 200 c c sea-water 4 Sea-water 5 100 c c sea water + 10 c.c. 2½M NaCl.

Table II

No of solution (see Table I)	Osmotic pressure in atmospheres	Time required: For healing wound	Time required: For perforation of mouth	Course of regeneration
4	2·0	Wounds never close	—	All pieces die under 21 days Both gonads show degeneration Gut cells swollen and vacuolated
5	3 5	8–10 days	Mouth never perforates	Testes continue functioning for 24 days after section, forming accumulations of sperm Gut cells vacuolated. Regeneration never complete
6	7 5	6–10 days	18 days	Testes function for 6 weeks after section Disintegration of gonads begins in 8 weeks Regeneration complete in 12 weeks
7	11 0	6–8 „	14 „	Testes only function for a few days after section Gonads very reduced in 8 weeks
8	15·0	5 7 „	10 „	Gut cells always remain normal Regeneration complete in 6 weeks Secondary sexual organs appear
9	18 5	3 „	8 „	Regeneration complete in 4–5 weeks Secondary sexual organs appear
10 (sea-water)	22 5	3–5 „	9 „	Regeneration complete in 7 weeks No trace of generative system present
11	29 5	6–9 „	21 „	Regeneration takes 8–9 weeks Genital system entirely absorbed
12	33 5	6–10 „	Mouth never perforates	Gut cells in these tissues appear shrunken, with very dense protoplasm. "Tailless" forms produced.
13	40 5	Wounds never close	—	All pieces die without any regeneration in 21 days.

The decrease in rate of regeneration can be measured proportionately by a consideration of the time taken to reach some definite stage. Two such points are considered here · (1) the time taken for the healing of the wound, (2) the time taken for the perforation of the new mouth These figures are summed up in Table II on p 11

It can be seen that regeneration is most rapid in solution 9 This solution consists of 100 c c distilled water + 500 c.c. sea-water. Regeneration in this solution is accompanied by reduction (absorption of the yolk-glands, etc.), but the regenerative processes proceed very rapidly, and, at the moment when the normal form is restored, reduction has not gone so far as to have removed the whole of the genital system Under these conditions the secondary sexual apparatus is redeveloped in the new tail This also occurs in solution 8 (100 c c. distilled water + 200 c c sea-water) In more hypotonic solutions, in sea-water and in hypertonic solutions, restoration of the normal form is a much slower process. In these solutions, the reduction due to regeneration + the reduction due to the longer starvation, make it impossible for the worm to redevelop the secondary sexual organs, even after the normal form has been restored.

In normal sea-water or hypertonic water, the removal of the posterior end of the worm acts as a check on the production of sperm Sperm present in the testicles at the time of section remains there, and, though the activity of the spermatocyte cells does not immediately cease, it is greatly diminished, and, as regeneration proceeds (under starvation conditions), the whole generative system is slowly absorbed to feed the growing parts In worms regenerating in hypotonic water, the production of sperm continues for some time longer, and the sperm produced leaves the testes, passes down to the cut end of the vas deferens, where, being unable to escape, it collects in sinuses, one on either side This condition is shown in fig 10

In figs 11 and 12, A and B, are shown camera drawings of gut cells (*g c*) of animals regenerating in hypo- and hypertonic water, and also, for comparison gut cells of normal whole animals In the strongly hypotonic solutions the cells are swollen and vacuolated, and the cell boundaries are hard to distinguish. In the hypertonic solution the cells are shrunken This fact suggests that in the one case water has actually been absorbed by the tissues, and in the other case extracted from them. From this it appears that the epidermis in *Gunda* must be a highly protective membrane, since whole animals placed in similar media show no such changes.

A histological study of the tailless forms produced at 33 5 atmospheres was made by sectioning the worms at various stages These sections show certain peculiarities in the behaviour of the parenchyma cells. In sea-water after

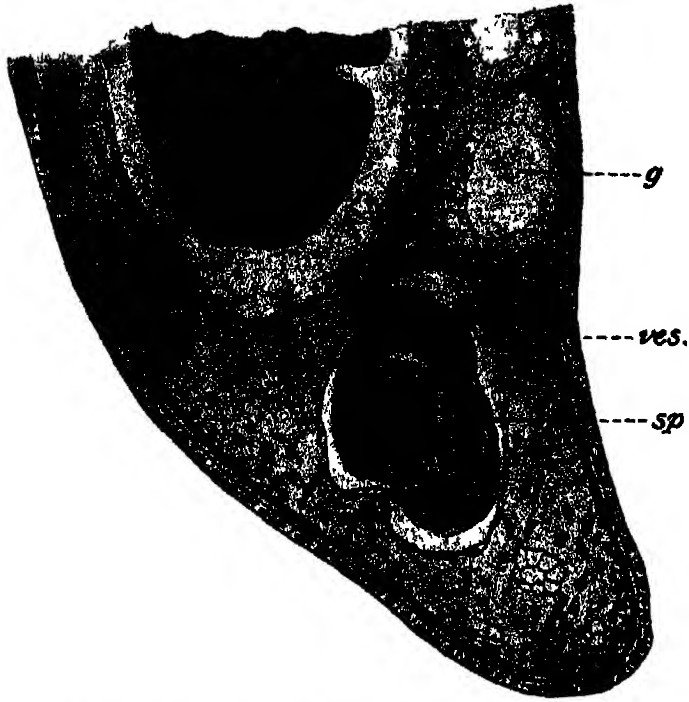


FIG 10.—Regeneration in 100 c.c. distilled water + 50 c.c. sea-water 43 days
g, gut *sp*, sperm. *ves*, vesicle

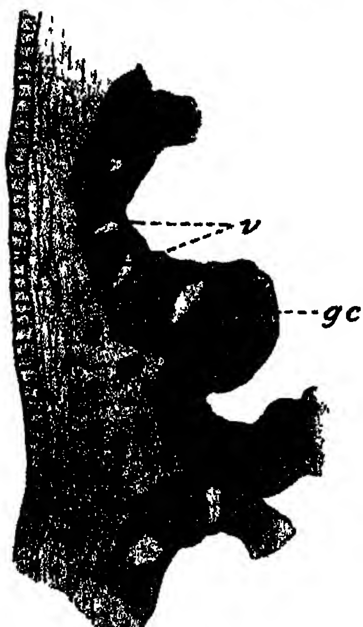
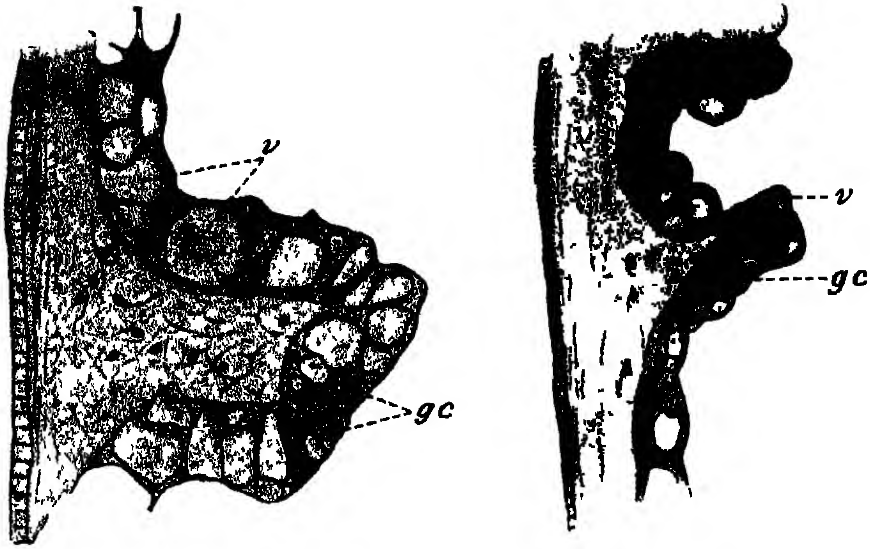


FIG. 11.—Normal gut cells.
g.c., gut cell. *v.*, vacuole



A B

Fig 12.

A —Gut cells from worm regenerating in 100 c c distilled water + 50 c.c sea-water 43 days

B —Gut cells from worm regenerating in 100 c c sea-water + 20 c.c. 2½M NaCl 32 days.

g c, gut cell. *v*, vacuole

section, the parenchyma cells congregate in the region of the wound (see above), but in the solution of high osmotic pressure they only travel to the wound very slowly The first result of this slow migration appears in the greater length of time required to heal the wound (6–10 days as compared with 3–5 days in sea-water). The next point to be noticed is that the parenchyma cells do not collect together at the hind end to form the mass which is subsequently to become the new tail (figs. 13 and 14) These tailless forms never develop the perforation for the new mouth They develop a thin muscle layer under the new epithelium, but the circuit of the nervous system is not restored From the third week after section they develop large vesicular spaces (see fig 8, 7 and 8, *v*) in the gut, or in the pharynx chamber, after 7–8 weeks these become filled with masses of disintegrating tissue, and the animals subsequently die. A similar retardation of the movements of the parenchyma cells is found in the strongly hypotonic media.

This checking in the processes of restoration is also to be found to a parallel degree in the processes of reduction. For instance, in sea-water an anterior half is completely restored in 8 weeks, and during restoration every

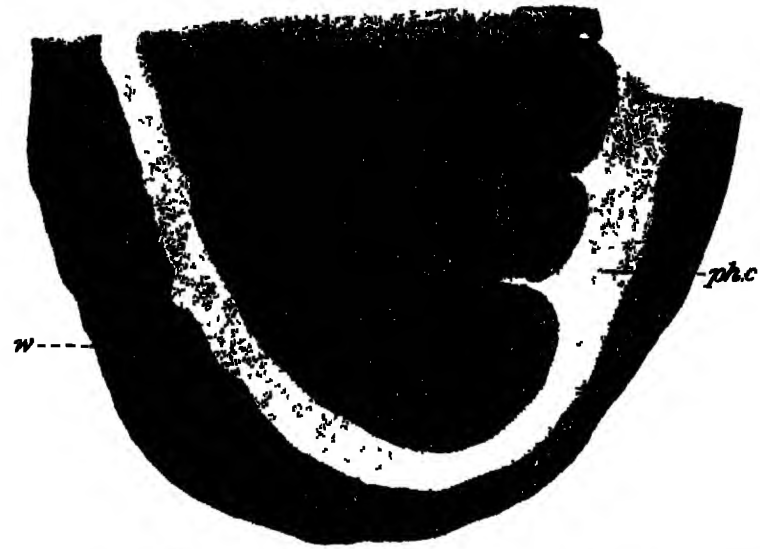


FIG 13.—Regeneration in 100 c.c. distilled water + 50 c.c. sea water 15 days *ph.c*, pharynx chamber *w*, point of closure of wound

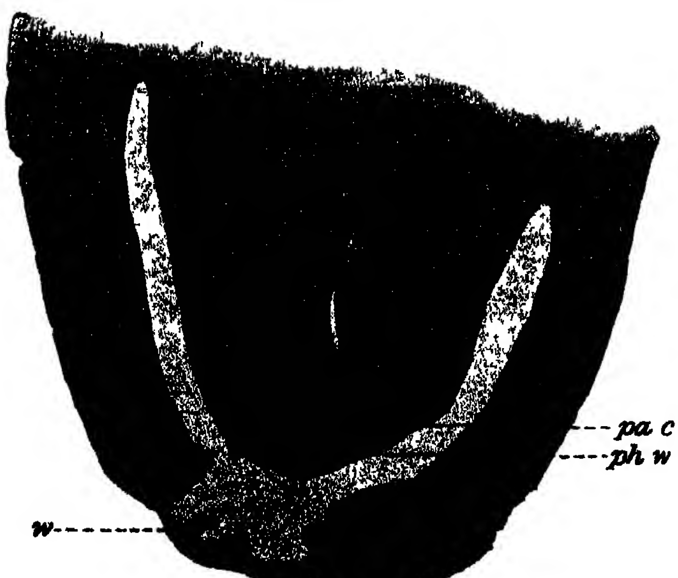


FIG. 14.—Regeneration in 100 c c sea-water + 50 c.c 2½M NaCl. 6 days. *pa.c.*, parenchyma cell. *ph. w*, point of closure of pharynx wound *w*, point of closure of wound.

trace of the genital system is absorbed. In the tailless forms 8 weeks after section it is found that the genital system is not reduced to any great extent

The slight degree to which the reduction processes are carried is reflected in the external form of these pieces, which only suffers very slight changes during the time (*cf.* figs. 5 and 15).

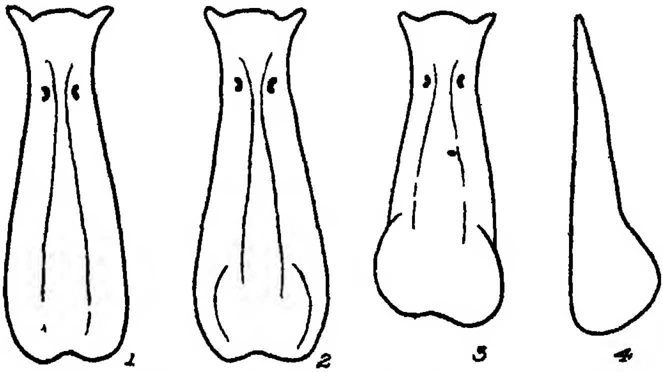


FIG 15.—Stages of Regeneration in 100 c.c. sea-water + 20 c c 2½M NaCl
1 10 days. 2 14 days. 3 28 days 4 Side view of 3.

Many sections were examined for mitotic figures, and a comparison was made between the mitoses found in worms regulating in sea-water and in water with a higher or lower osmotic pressure Text-fig 16 shows the type of mitoses found. At osmotic pressures of 7 5 and 33 atmospheres the mitoses appear to be a little irregular, but in all cases mitotic figures are so rare that it would not be justifiable to ascribe a principal role in the failure to regenerate, to any such irregularities.

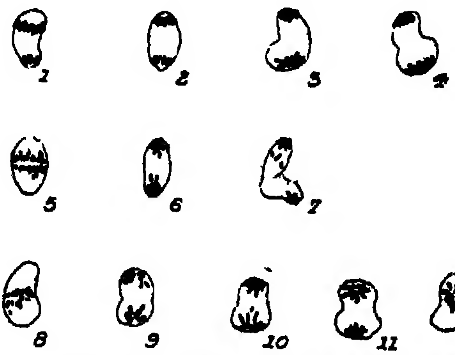


FIG 16.—Mitotic Figures from the Parenchyma Cells.

1–4. From tissue regenerating in sea-water 5–7 From tissue regenerating in 100 c.c. distilled water + 50 c c. sea-water 8–12. From tissue regenerating in 100 c.c. sea-water + 20 c c. 2½M NaCl.

VI Discussion.

The results given in this paper show that for *G ulvæ* there is a definite value for the osmotic pressure of the surrounding medium, at which regeneration of the hind end occurs most rapidly This point lies inside the range of variation of osmotic pressure to which the animals are subjected in their natural environment Above or below this point the rate of regeneration diminishes, until finally it is entirely inhibited A similar phenomenon has been obtained by Child (2) in *Planaria* by adding doses of alcohol, ether, and potassium cyanide to his cultures, or by raising the temperature to a point just below that which causes the death of the tissues Child has ascribed this change of rate of regeneration, with its final production of tailless forms, to the lowering action of the external agent employed upon the "rate of action" of the regenerating fragment as a whole

In the present case the morphological cause of the reduced rate of regeneration can be localised in the parenchyma cells These normally migrate in large numbers to the region of a wound and there build up the new tissues When, however, the osmotic pressure of the surrounding medium is removed from the optimum value, this migration is checked and finally inhibited In the latter case, the wound never heals, and the fragment ultimately dies. In the case where the osmotic pressure is just short of completely preventing the migration of the cells, the wound slowly closes but no new organs are formed This produces the so-called tailless forms. The swelling that occurs at the hind end in these cases is not due to the formation of tissue but to the development of large cavities in the gut or pharyngeal chamber, or, in hypotonic media, to the absorption of water

In *Planaria maculata*, Curtis (3) describes the development and absorption of the genital system as a regular seasonal change *G ulvæ*, however, is found sexually mature at Plymouth all the year round Once it has reached maturity, the genital system is only re-absorbed under conditions which make it necessary for the animals to use their own tissues as a source of energy. These conditions are—(1) hunger, (2) regeneration after a cut which has removed a fairly large proportion of the animal's body (Doubtless, after loss of small amounts, some reduction takes place, but not enough to be easily recognised) In the regulation of *G. ulvæ*, after a loss of some of its parts, reduction and regeneration go hand in hand, and in some cases where regeneration is inhibited by some external factor, *e g*, high osmotic pressure, reduction is inhibited to the same extent

As Stockard and others have already pointed out, regenerating parts have a potent influence on the old body This fact is again well illustrated in

the sections shown in fig. 8. It can be seen that where a new part is growing, an old part is correspondingly diminishing (fig. 8, 1–4). In cases where there is no formation of new parts (fig. 8, 5–8), there is correspondingly no diminution of the old ones. Thus, when no tail grows out, and where the pharynx also shows no growth, the part anterior to the pharynx remains with unchanged proportions. The value of the irregular mitoses found in the parenchyma cells under the conditions unfavourable to development remains to be considered. Under normal conditions regeneration takes place without the parenchyma cells increasing to any great extent in number. For this reason it seems probable that the failure to restore the lost parts under conditions of very high or very low osmotic pressure is not due to failure of the parenchyma cells to divide, but that both checked migration and irregular mitoses are an expression of unfavourable change in the physiological conditions. This suggestion receives support from the appearance presented by the gut cells, which in strongly hyptonic solutions are swollen and vacuolar, while in hypertonic solutions they are contracted and dense.

The most favourable conditions for the regeneration of *G. ulvæ* are in a mixture of 100 parts of distilled water to 500 of sea-water, and at an osmotic pressure of 18 atmospheres. Regeneration occurs, though less rapidly, in solutions having an osmotic pressure as low as 7.5, or as high as 29.5 atmospheres. Above or below these two points the water content of the tissues appears to have been altered to such an extent that the cells, notably the parenchyma cells, can no longer perform their normal functions.

Summary.

1. *G. ulvæ* is capable of living indefinitely in water having an osmotic pressure of more than 5 and less than 33 atmospheres.

2. The rate of regeneration of the posterior end in *G. ulvæ* depends on the osmotic pressure of the medium. This osmotic pressure has an optimum value for regeneration at 18 atmospheres, *i.e.*, just below that of sea-water, and limiting values at 5 and 33.5 atmospheres.

3. Restoration of lost parts in *G. ulvæ* is brought about entirely by the undifferentiated parenchyma cells which migrate to the region of the wound and build up the lost parts.

4. For values of the osmotic pressure lying between the optimum and the limiting values, this migration of the parenchyma cells is retarded, and the rate of restoration is retarded to a similar degree. At the limiting values of the osmotic pressure there is no migration of the parenchyma cells and no restoration of lost parts.

5 Under starvation conditions, *G. ulvæ* undergoes reduction. This consists in (1) absorption of the genital system, (2) general reduction in size Both these are brought about by the activity of the parenchyma cells

6 During the process of restoration of lost parts the same reduction processes occur as in starvation Where the restoration of lost parts is retarded, *e g*, by raising or lowering the osmotic pressure, reduction is retarded to precisely the same extent

7 In sea-water or hypertonic solutions removal of the posterior half of the body inhibits further production of sperm In hypotonic solutions sperm continues to be produced for a varying length of time

8 In strongly hypertonic solution examination of the gut cells shows that these have diminished in size and become more dense In strongly hypotonic solutions they have increased in size and become vacuolar

I should like to take this opportunity of thanking the director and staff of the Plymouth laboratory for the promptness with which they have supplied me with material, and for their unfailing kindness during the whole time I have worked at the laboratory I should like also to acknowledge my indebtedness to the Royal Society, the Zoological Society, and the University of Cambridge for the use of their tables at Plymouth Finally, I wish to thank the Trustees of the Balfour Fund for a grant which made it possible for me to take up the work in 1912

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Glossina brevipalpis *as a Carrier of Trypanosome Disease in Nyasaland*

By Surgeon-General Sir David Bruce, C.B., F R S, A M S., Major A E Hamerton, D S O, and Captain D P Watson, R A M C; and Lady Bruce, R R C (Scientific Commission of the Royal Society, Nyasaland, 1912-14)*

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[Plate 1]

Introduction

Glossina brevipalpis (Newstead) is found at many spots along the west shore of Lake Nyasa The nearest point to the Commission's camp at Kasu where they were at all common was at the mouth of the Lingadzi river (13° 27′ S lat, 34° 19′ E long). This was 50 miles away, but with the aid of a motor-cycle specimens of these flies were brought up to the camp

It is proposed in this paper to give (I) a short account of the habits of this tsetse fly, (II) the results of the dissection of the flies, (III) the infectivity of the wild flies, and (IV) the result of various transmission experiments

One of the members of the Commission camped on the Lake-shore from April 26 to May 10, 1913, to superintend the catching and sending to the camp of these flies He has supplied the following account of their habits.—

* Major D Harvey, R.A.M C, took part in the work described in this and the four following papers, but, having left the Commission in September, 1913, before the reports were drawn up, his name does not appear in the titles.

I Habits of G brevipalpis

G. brevipalpis is found frequenting the roads in a small area of country around Markho village on the west shore of Lake Nyasa, at the mouth of the Lingadzi river This tract of country, which may be roughly estimated at about 5 square miles in extent, is broken up by swampy hollows and streams forming the delta of the Lingadzi It is covered with palm forest and a dense undergrowth of high grass and bush, and is traversed by two main roads, one between Markho and Lingadzi estate running east and west, and another running parallel to the Lake-shore and about 1 mile to the west of it. The roads are hoed tracks about 3 yards broad, cut through the palm forest and walled in by high grass and almost impenetrable bush

G brevipalpis is crepuscular in its habits and quite unlike other Nyasaland tsetse flies During the daytime, from dawn till about an hour before dark, one may pass along the roads or wander in the surrounding jungle without encountering a single fly of this species But as evening approaches odd flies suddenly appear sitting motionless in the middle of the hard-trodden path all the way along the road between Markho and Kasache, a distance of 2 miles, and for about 2 or 3 miles up the Lingadzi road They do not follow or settle upon passers-by like other tsetse flies, and they would pay no attention to a dog which was repeatedly walked through their haunts in the evening In the dim light they are difficult to see, and resemble little bits of earth on the path, but the searcher's attention is attracted by the sound of them buzzing up as they are disturbed by his footsteps They immediately settle on the path again and are easily caught, for if missed by the first stroke of the net they at once resettle near the same spot The hard-trodden surface of the path seems to have an irresistible attraction for them They do not move about in search of food or chase each other, but remain motionless for several minutes, and when they move it is only to fly up and immediately settle again in the middle of the path They were never seen on the roads at dawn, as the mornings at this time of the year were invariably cold and misty

Out of the 500 flies caught and examined on the spot all were males Some of them were found on dissection to contain mammalian blood Game, such as buffalo and several species of antelope, is common in the district

Flies kept in captivity remain dormant on the sides of their cage during the day At night they are very active and buzz incessantly in their efforts to escape. If the side of the cage be applied to the skin of a goat or a dog they will feed with avidity at any time of the day or night

A few wild flies were dissected in order to ascertain their natural food.

In 50 flies seven contained recognisable blood This in six cases was antelope blood, in the seventh probably human

It is curious the great preponderance of males over females. It seems to be a habit of the former to frequent paths in the evening, while the females presumably hide in the thick jungle The same thing obtained to a lesser extent with *G. morsitans.* Among all the *G. brevipalpis* dissected at the laboratory, amounting to several hundreds, only four females were found

II Dissection of Wild G brevipalpis to Ascertain with what Species of Trypanosomes, if any, they are Infected

Four hundred and ninety-six wild flies were examined, 44 of these were positive and 452 negative Table I gives the result of the dissection of the positive flies

Among the 44 flies which contained flagellates, it was possible to make a more or less correct guess at the species in 19 These are *T brucei vel rhodesiense*, 1, *T pecorum*, 9, *T simiæ*, 1, and *T grayi*, 8 In 10 flies the flagellates were considered to belong to a pathogenic type, species unknown No opinion could be expressed about the remaining 15

It is curious that *G brevipalpis* should contain flagellates resembling *T. grayi*, which is so often found in *G palpalis* Now one thing common to *G brevipalpis* and *G palpalis* is that they both live alongside water. This would point to the vertebrate host of *T grayi*, if there is one, being some water animal, such as the crocodile or iguana, or some water bird.

Conclusion.

Wild *G brevipalpis* are naturally infected with *T. brucei vel rhodesiense*, *T pecorum* and *T simiæ*.

Table I

Date	No of fly	Proboscis: Labial cavity	Proboscis: Hypopharynx	Proventriculus	Crop.	Fore-gut.	Mid-gut.	Hind-gut	Salivary glands
1912									
June 14	1	−	−				+		−
,, 14	2	−	−	−	−	−	+ +	+ +	
,, 14	3	+				+	+		
,, 14	4	−	−		+ +	+ +	+ +		
,, 28	5	−	−				+		−
,, 28	6	−	−			+	+		
,, 28	7	+				+ +	+ +		
,, 28	8	−	−			+ +	+ +		
,, 28	9	−	−			+ +	+ +		
Sept 12	10	−	−	+ +		+ +	+ +		
1913									
Mar 5	11	+	+ +			+ +	+ +	+ +	−
,, 18	12	−	−				+ +	−	−
,, 18	13	−	−			+ +	+ +	+ +	−
,, 21	14	−	+	−		−	+		−
April 4	15	−	−	−		+	+	+	−
,, 22	16	−	−				+		
May 22	17						+		
,, 31	18	+				+ +	+ +		
,, 31	19	−	−				+		−
June 2	20	−	−				+		−
,, 2	21	+				+	+		−
,, 5	22	+ +	+ +			+ +	+ +	+ +	
,, 9	23	−	−				+		
,, 12	24	+ +	+ +	+ +		+ +	+ +	+ +	−
,, 23	25	−	−			+ +	+ +	+ +	+ +
,, 24	26	+ +	+ +			+ +	+ +	+ +	
,, 25	27	−	−				+		−
,, 26	28	−	−				+		−
,, 26	29	+				+	+		−
July 1	30	+ +	+ +			+ +	+ +	+ +	
,, 5	31	−	−				+		−
,, 11	32	−	−				+		−
,, 14	33	+ +	−			+ +	+ +		−
,, 14	34	−	−				+		−
,, 14	35	+ +	−			+ +	+ +		
,, 16	36	−	−				+		−
,, 16	37	+	−			+ +	+ +		−
,, 16	38	+ +	+			+ +	+ +		−
,, 24	39	−	−				+		−
,, 24	40	+					+		−
,, 24	41	−	−				+		−
,, 24	42	−	−				+		−
,, 30	43	+	−				+		−
,, 30	44	−	−				+		

III. The Infectivity of the Wild G. brevipalpis

A few experiments were made to test the natural infectivity of *G. brevipalpis* by feeding them on healthy animals

When the flies were brought up from the Lake-shore they were fed on a

monkey, dog, and goat Five experiments in all were made, four negative, one positive. The following Table gives the result :—

Table II.—Feeding Wild *Glossina brevipalpis*

Date	No of flies fed	Monkey				Dog				Goat			
		T brucei vel rhodesiense	*T pecorum*	*T simiæ*	*T capræ*	*T brucei vel rhodesiense*	*T pecorum*	*T simiæ*	*T capræ*	*T brucei vel rhodesiense*	*T pecorum.*	*T simiæ*	*T capræ*
1912 June 14	42	—	—	—	—	—	—	—	—	—	—	—	—
1913 Mar 18	146	—	—	—	—					—	—	—	—
Apr 29	541	—	+	—	—	—	+	—	—	—	+	—	—
May 7	90	—	—	—	—								
June 25	276	—	—	—	—	—	—	—	—	—	—	—	—
Total	1095												

IV Transmission Experiments

Several experiments were carried out with *G brevipalpis*, to ascertain if this species of tsetse fly can act as a carrier of the various pathogenic trypanosomes found in Nyasaland

These experiments were made, not with laboratory-bred but with wild flies, and this of course takes away much of their value It was found impossible, on account of the distance from the Lake-shore and the scarcity of flies, to attempt the breeding of *G brevipalpis*, in order to obtain laboratory-bred flies

The species of trypanosomes experimented with were · (1) *T. brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland, (2) *T brucei*, Zululand, 1913, (3) *T. pecorum*, and (4) *T. capræ*.

1. *The Development of* T. brucei vel rhodesiense *in* G brevipalpis.

(a) *Feeding Wild* G. brevipalpis *first on Animals infected with the Trypanosome causing Disease in Man in Nyasaland and then on Healthy Animals, to discover if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly*

Two hundred and thirty-two wild *G. brevipalpis* were used in six experiments, but in no case with a positive result. Only fifty-three flies were dissected and eight infected flies found. If all had been dissected probably about 30 to 40 infected flies would have been found.

Table III

Date.	Expt.	No of flies used.	Expt positive or negative	No of flies dissected	No of infected flies found	No of days before flies became infective
1918.						
Mar 6	1986	2	—	1	0	
„ 26	2062	10	—	6	0	
June 2	2201	70	—	8	2	
„ 9	2218	60	—	19	2	
„ 21	2232	50	—	18	4	
July 23	2310	40	—	1	0	

(b) *Details of the Six Negative Experiments*

Table IV.

Expt	Day of expt	Procedure	Remarks
1986	1 2–4 5–15	Flies fed on infected Guinea pig 1658 Starved Fed on clean Guinea pig 1907.	Only 2 flies used, one escaped and the other died on the 15th day of the experiment, on dissection it was found negative
2062	1–4 5 6–28	Flies fed on infected Monkey 970 Starved Fed on clean Dog 2041	10 flies used 6 were dissected, all negative
2201	1–6 7 8–33	Flies fed on infected Monkey 2156 Starved Fed on clean Dog 2211	70 flies used Only 8 were dissected, 2 found infected, one with *T grayi*, the other with a pathogenic type of trypanosome
2218	1–12 13–14 15–44	Flies fed on infected Monkey 2151 Starved Fed on clean Dog 2237	60 flies used 2 found infected Only 19 dissected
2232	1–6 7–9 10–42	Flies fed on infected Monkey 2152 Starved. Fed on clean Dog 2244	50 flies used, 4 found infected 18 flies dissected The 4 infected flies showed infection of the gut alone
2310	1–3 4–5 6–39	Flies fed on infected Monkey 1792 Starved Fed on clean Dog 2315	40 flies used Only one fly dissected, negative

(c) *Result of the Dissection of the Infected Flies.*

As will be seen from Table III, eight infected flies were found among the *G. brevipalpis* which had fed on animals infected with the trypanosome causing disease in man in Nyasaland. The following Table gives the result of the dissection of these eight flies:—

Table V

Expt.	Time, days	Proboscis	Alimentary tract	Salivary glands
2201	30	−	+	−
2201	37	−	+	−
2213	33	−	+	+ + +
2213	46	+	+ +	−
2232	37		+	−
2232	37		+	−
2232	44	−	+	−
2232	44	−	+	−

In Experiment 2201 there were two infected flies found One of these appeared to be infected with *T grayi*, the other with a trypanosome of a pathogenic type

In Experiment 2213 there were also two infected flies In one the development was restricted to the proboscis and the alimentary tract, in the other the salivary glands as well as the intestine were found to be swarming with trypanosomes This fly, which was dissected 33 days after it had fed on an infected monkey, had the whole lumen of the glands filled with active motile trypanosomes, which came pouring out of the broken end of the glands It was thought at the time that these must be infective forms of the trypanosome causing disease in man in Nyasaland, but a part of the salivary glands and contents of gut injected into a white rat failed to infect it In spite of this negative experiment, however, it is probable that this represents a true development of the Nyasaland trypanosome in *G. brevipalpis*, the development not having reached the infective stage. A fuller description of the morphology of these salivary forms will be given under the next heading It may be noted here that salivary glands infected by *T brucei vel rhodesiense* or by *T brucei*, Zululand, 1913, seem to be more crowded with trypanosomes than in the corresponding infection of *G palpalis* by *T gambiense* The salivary glands in the former case appear to be swollen and bursting with the flagellates.

In Experiment 2232 there were four infected flies found, but in none was there any invasion of the salivary glands.

(d) *Morphology of the Trypanosomes found in the Salivary Glands of a Wild* G. brevipalpis *which had fed on a Monkey infected with the Trypanosome causing Disease in Man in Nyasaland.*

This fly, as described above, was found in Experiment 2213, and failed to infect the clean Dog 2237 which it had been fed upon. Part of the salivary

glands and contents of the intestine also failed to infect Rat 2234. It would appear, then, that this fly had not yet reached the infective stage

It is now proposed to describe the different forms of the trypanosomes found in the salivary glands of this fly somewhat in detail, and to bring forward a theory in regard to a stage which seems to occur in the final development of this trypanosome in the salivary glands. The different forms and apparent stages in their development could be more easily made out in *G brevipalpis* than in *G morsitans,* on account of its greater size On Plate 1 this evolution of the trypanosome causing disease in man in Nyasaland in the salivary glands is represented

Figs. 1 and 2 represent the long, slender developmental forms of trypanosomes found in the intestine of the fly, from the mid-gut to the proventriculus. It is this type of trypanosome which invades the salivary glands

Fig 3 shows the change in shape which the intestinal forms undergo on entering the salivary glands The posterior extremity lengthens somewhat and the micronucleus and flagellum pass forward This appears to be the commencement of the change to the crithidial type

Fig 4 represents the fully developed crithidial form of the parasite The micronucleus and flagellum have passed further forward until they lie anterior to the nucleus The anterior portion of the parasite has broadened out, the posterior has become attenuated

Fig 5, the parasite is still crithidial in form The anterior half has become still broader, the posterior half elongated and further attenuated

Fig 6, the attenuated posterior portion has shortened

Figs. 7, 8 and 9 represent further stages in the evolution The long attenuated posterior extremity has disappeared and the parasite has become contracted and thickened

Fig 10 shows the last phase of the crithidial stage. Here the parasite has assumed a rounded form and the flagellum is folding on itself.

Fig. 11 represents a group of parasites in the encysted stage In this form they are found massed together in the lumen of the salivary glands

Fig. 12, in this group the encysted forms are just unfolding

Fig. 13 shows a single encysted form breaking open. The micronucleus is now posterior to the nucleus; the crithidial has become the trypanosomal

Figs. 14, 15, 16, and 17 demonstrate further stages in the unfolding of the encysted form. The parasite is now assuming a trypanosome shape

Figs. 18 and 19 show the fully developed salivary-gland form of the trypanosome This constitutes a reversion to the "blood form" from which the cycle of development began and is the only infective form

On comparing these figures with the developmental forms of the

trypanosome causing disease in man in Nyasaland in *G. morsitans*,* or with the same forms of *T brucei*, Zululand, 1913,† it will be apparent that in all probability this is a true development of this trypanosome in *G. brevipalpis* It is true wild flies are being dealt with, but in this district it is only trypanosomes of this type which invade the salivary glands, so that *T pecorum*, *T simiæ*, and *T. capræ* are excluded It is very unlikely that *T. grayi* invades the salivary glands

Conclusion

G brevipalpis is capable of acting as a carrier of *T brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland

2. *The Development of* T brucei, *Zululand*, 1913, *in* G brevipalpis

(a) *Feeding Wild* G. brevipalpis *first on Animals infected with* T brucei, *Zululand*, 1913, *and then on Healthy Animals, to ascertain if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly*

Table VI

Date	Expt	No of flies used	Expt positive or negative	No of flies dissected.	No of infected flies found	No of days before flies became infective
1912 May 12	2130	80	–	10	1	
July 1	2250	60	+	2	0	51
„ 17	2299	60	–	0	0	

(b) *Details of the Three Experiments*

Table VII

Expt	Day of expt	Procedure	Remarks
2130	1-2 3 4-85	Flies fed on infected Monkey 1970 Starved Fed on clean Dog 2142	80 flies used; 1 found infected; only 10 dissected.
2250	1-4 5-6 7-60	Flies fed on infected Dog 2240 Starved Fed on clean Dog 2276.	60 flies used; only 2 dissected, both negative Dog 2276 showed trypanosomes on the 58th day.
2299	1-10 11 12-61	Flies fed on infected Dog 2254. Starved. Fed on clean Dog 2314.	60 flies used; none dissected

* 'Roy. Soc. Proc.,' B, vol. 87, p. 516 (1914).
† *Ibid.*, B, vol 87, p. 493 (1914).

Two hundred wild *G. brevipalpis* were used in three experiments—one positive, two negative. Only twelve flies were dissected, one of which was found to contain in the intestine long, ribbon-like trypanosomes of apparently a pathogenic type, but it was not possible to place it It is to be regretted that all the flies had not been dissected as was the rule in these transmission experiments.

Dog 2276 became infected 58 days after the flies had fed on the infected animal. This would point to the development of *T brucei*, Zululand, in the flies If there had been a fly in the cage naturally infected with the Nyasaland strain, then the animal fed upon ought to have shown trypanosomes in its blood earlier than 58 days It may therefore be held as highly probable that Dog 2276 was infected by a *G brevipalpis* in which a development of *T brucei*, Zululand, 1913, had taken place

Conclusion

G brevipalpis is capable of acting as a carrier of *T. brucei*, Zululand, 1913

3. *The Development of* T pecorum *in* G. brevipalpis

(a) *Feeding Wild* G brevipalpis *first on Animals infected with* T pecorum *and then on Healthy Animals, to ascertain if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly*

Table VIII

Date.	Expt.	No of flies used	Expt positive or negative	No of flies dissected	No of infected flies found	No of days before flies became infective
1912 May 28	2190	80	+	17	6	29
June 2	2207	50	–	16	0	

One hundred and thirty wild *G. brevipalpis* were used in two experiments—one positive, one negative Only 33 flies were dissected, six found infected

(b) *Details of the Two Experiments.*

Table IX.

Expt.	Day of expt.	Procedure	Remarks.
2190	1–3 4–5 6–86	Flies fed on infected Goat 2186. Starved. Fed on clean Goat 2202	80 flies used; 6 infected flies found; only 17 dissected Goat 2202 showed trypanosomes on the 36th day
2207	1–6 7 8–46	Flies fed on infected Goat 2186 Starved. Fed on clean Goat 2210	50 flies used; only 16 flies dissected, all negative.

(c) *Result of the Dissection of the Infected Flies*

Table X

Expt	Time, days	Proboscis.		Alimentary tract	Salivary glands
		Labial cavity	Hypopharynx		
2190	23	—	—	+	
2190	23	+	+ +	+	
2190	36	—	—	+	—
2190	36	+	+ +	+	—
2190	36	+	+ +	+	—
2190	36	+	+ +	+	—

In Experiment 2190 six infected flies were found In four of these the hypopharynx was blocked with small "blood forms" of *T pecorum* Taking into consideration the time which elapsed between the feeding on the infected goat and the appearance of an infective fly in the cage—23 days—and, further, the number of flies found infected with *T. pecorum*—4 in 33—it must be admitted that a development of *T pecorum* has taken place in *G brevipalpis*

Conclusion

G brevipalpis is capable of acting as a carrier of *T pecorum*

4. *The Development of* T capræ *in* G brevipalpis

(a) *Feeding wild* G brevipalpis, *first on Animals infected with* T capræ, *and then on Healthy Animals, to ascertain if this Species of Trypanosome passes through a Cycle of Development in this Species of Tsetse Fly.*

Three experiments were carried out with wild flies One was negative and two positive. On examining the positive experiments, they were found to be *T pecorum* infections, and not *T. capræ* The animals were probably infected by naturally-infected *G brevipalpis.* No infection by *T capræ* took place, but one of the flies, on dissection, was shown to have an undoubted development of *T capræ* in the labial cavity and hypopharynx.

Table XI

Date.	Expt	No of flies used	Expt positive or negative	No of flies dissected	No of infected flies found.	No of days before flies became infective.
1912						
April 11	2071	11	—	3	1	
May 30	2199	60	+	25	1	30
July 17	2277	50	+	0	6	24

(b) *Details of the Three Experiments*

Table XII

Expt	Day of expt	Procedure	Remarks
2071	1–8	Flies fed on infected Goat 1912	11 flies used; 1 infected fly found
	9–10	Starved	Only 8 dissected
	11–12	Fed on clean Goat 2103	
2199	1–8	Flies fed on infected Goat 1912	60 flies used, 1 infected fly found
	9	Starved	25 dissected Goat 2212 showed
	10–28	Fed on clean Goat 2212	trypanosomes on the 27th day
	29–30	Starved.	Goat 2245 negative
	31–50	Fed on clean Goat 2245	
2277	1–5	Flies fed on infected Goat 2220	50 flies used. No flies dissected
	6–7	Starved	Goat 2387 showed trypanosomes on
	8–32	Fed on clean Goat 2287	the 31st day, and Goat 2362 on the
	33–34	Starved	49th day
	35–49	Fed on clean Goat 2362	

(c) *Result of the Dissection of the Two Infected Flies*

Table XIII.

Expt	Time, days	Proboscis		Alimentary tract	Salivary glands
		Labial cavity	Hypopharynx		
2071	9	+	+ +	–	–
2199	45	+ +	–	+ + +	

In Experiment 2071 there is evidently a development of *T capræ* in the fly found infected. The intestine and salivary glands are free, whereas the hypopharynx is crammed with numerous short trypanosomes of the *T capræ* type. In the labial cavity one large colony of large flagellates of a crithidial type was seen

In Experiment 2199 the intestine and labial cavity of the infected fly were found to have a heavy infection of trypanosomes As the intestine was also involved, this is probably a natural infection of the fly with *T. pecorum* The animal the flies were fed on was found to be suffering from a *T pecorum* infection

In the third experiment—2277—none of the flies were dissected, but, as the animal the flies were fed on became infected, as in the last experiment,

with *T. pecorum*, and not with *T. capræ*, it is probable that no complete development of the latter had taken place

Conclusion.

G. brevipalpis is capable of acting as a carrier of *T. capræ.*

General Conclusion

G brevipalpis is capable of acting as a carrier of *T brucei vel rhodesiense*, *T brucei*, Zululand, 1913, *T. pecorum*, and *T capræ*

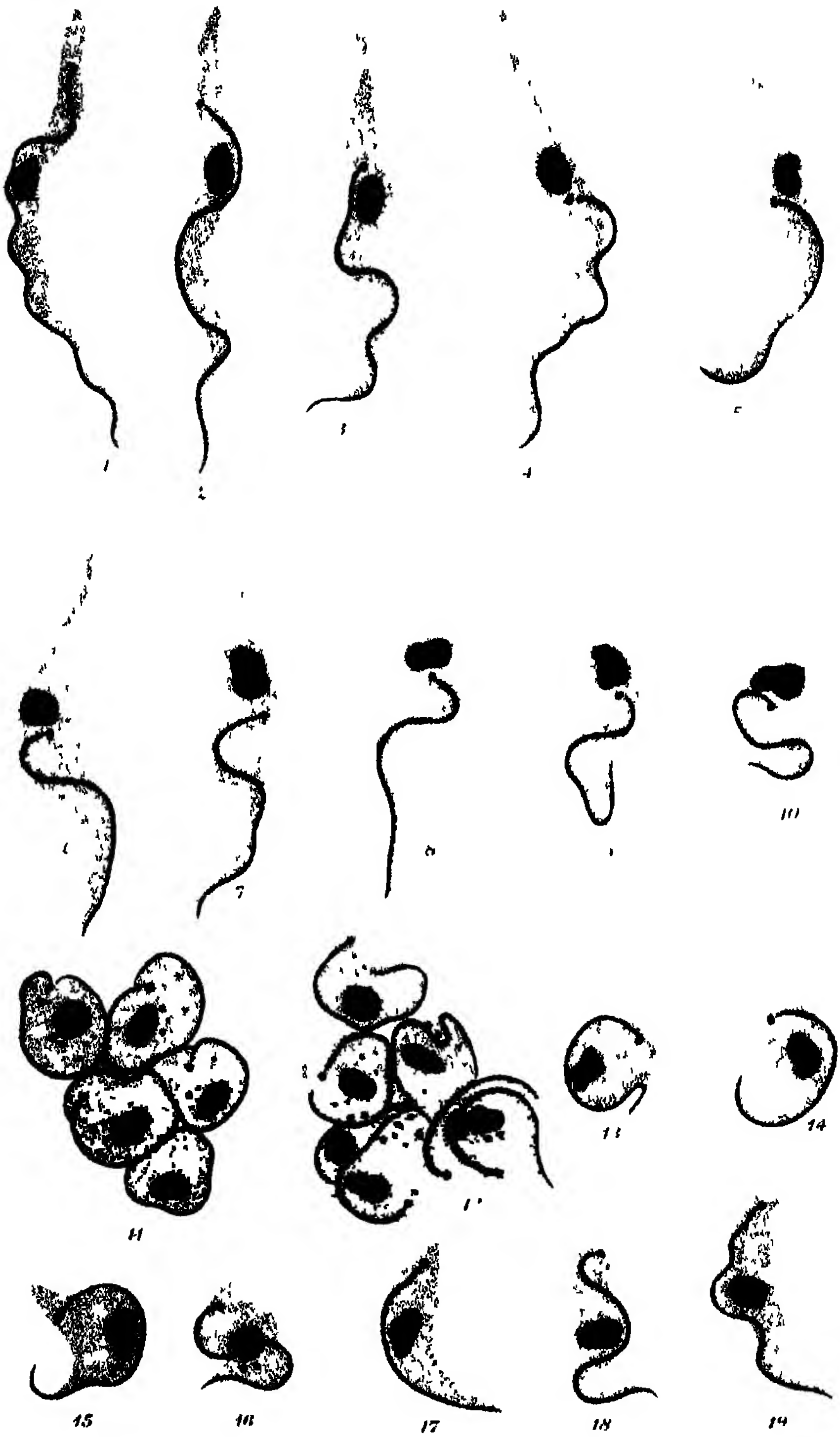
DESCRIPTION OF PLATE

(See also p 27 above)

Plate 1, *Trypanosoma brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland.

Figs 1 and 2, intestinal forms
Fig 3, intestinal form after entering salivary glands
Figs. 4–10, crithidial forms.
Figs 11 and 12, groups of parasites in the encysted stage
Figs 13–17, encysted forms opening out.
Figs 18 and 19, fully developed salivary-gland forms This constitutes the end of the cycle of development, which ends where it began, in the "blood form" of the vertebrate host.

Stained Giemsa. × 2000



Trypanosome causing Disease in Man in Nyasaland
Development in Glossina brevipalpis

A Gibbons del *X 2000*

Trypanosome Diseases of Domestic Animals in Nyasaland. III. — Trypanosoma pecorum. *Development in* Glossina morsitans.

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S., Major A. E. HAMERTON, D.S.O., and Captain D. P. WATSON, R.A.M.C.; and LADY BRUCE, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received March 25,—Read April 30, 1914.)

[PLATE 2.]

INTRODUCTION.

In a previous paper* the morphology of this species of trypanosome and its action on animals were described. In this it is intended to give an account of its development in *Glossina morsitans.*

This trypanosome belongs to the group in which the development takes place first in the gut, then passes forward into the labial cavity of the proboscis, and finally reaches the hypopharynx, where the trypanosomes revert to the original "blood-forms" and become infective. There is no infection of the salivary glands.

THE DEVELOPMENT OF T. PECORUM IN G. MORSITANS.

Seven experiments were carried out with laboratory-bred flies. Five were positive and two negative.

Table I.—Laboratory-bred Flies.

Date	Expt.	No. of flies used.	Expt. positive or negative.	No. of infected flies found.	No. of days before flies became infective.	Mean temperature.
1912						
May 16 .	848	22	+	4	53	69° F. (20·5° C.)
July 2 .	524	20	+	2	87	65° F. (18·3° C.)
1913						
Jan. 3 .	1732	60	–	0	—	84° F. (28·8° C.)
" 7	1787	40	+	3	19	84° F. (28·8° C.)
Feb. 10 .	1858	25	+	5	24	84° F. (28·8° C.)
" 24 .	1950	33	+	6	21	84° F. (28·8° C.)
April 29 .	2115	40	–	4	—	84° F. (28·8° C.)

* 'Roy. Soc. Proc.,' B, vol. 87, p. 1 (1913).

Two hundred and forty flies were used and twenty-four infected flies found—10 per cent. The first two experiments were carried out at the ordinary temperature of the laboratory, in the others the flies were kept in the incubator.

Details of the Five Positive Experiments.

The following Tables give the principal details in the carrying out of the five positive experiments They were all carried out with laboratory-bred flies —

Table II

Expt	Day of expt	Procedure	Remarks
546	1–3	22 flies fed on *T pecorum* infected dog	Goat 559 became infected on the 60th day; Dog 880 on the 82nd day All flies dissected; 4 found infected
	4	Starved	
	5–62	Fed on clean Goat 559	
	63	Starved	
	64–82	Fed on clean Dog 880	
524	1–5	20 flies fed on *T. pecorum* infected rat.	Trypanosomes first appeared in blood of Dog 541 on the 44th day. All flies dissected; 2 found infected
	6	Starved	
	7–44	Fed on clean Dog 541	
1737	1–3	40 flies fed on *T pecorum*-infected dog	Trypanosomes first appeared in blood of Dog 1750 on the 26th day All flies dissected; 3 found infected
	4	Starved	
	5–27	Fed on clean Dog 1750	
1858	1–3	25 flies fed on *T pecorum*-infected dog	Trypanosomes first appeared in blood of Goat 1903 on the 31st day 25 flies dissected; 5 found infected.
	4	Starved	
	5–25	Fed on clean Goat 1903	
1960	1–8	33 flies fed on *T. pecorum*-infected goat.	Trypanosomes first appeared in blood of Dog 1973 on the 28th day. All flies dissected; 6 found infected
	9	Starved	
	10–29	Fed on clean Dog 1973	

It would appear from these five positive experiments that a period of from 19 to 53 days may elapse before the cycle of development of *Trypanosoma pecorum* in *G. morsitans* is complete and the fly becomes infective.

Details of the Two Negative Experiments.

The following Table shows the method of procedure in carrying out the two negative experiments —

Table III

Expt	Day of expt	Procedure	Remarks.
1732	1–2 3 4–39	60 flies fed on *T. pecorum*-infected dog Starved. Fed on clean Dog 1736 (Experiment stopped)	Dog 1736 never showed trypanosomes 25 flies remained alive, used for another experiment Only 12 flies dissected; all negative
2115	1–4 5–6 7–8	40 flies fed on *T. pecorum*-infected rat. Starved Fed on clean Monkey 2066 (Experiment stopped)	Monkey 2066 never showed trypanosomes. All flies dissected, 4 found infected Experiment stopped on account of death of most of the flies

Result of the Dissection of the Infected Flies

The following Table gives the result of the dissection of the infected flies found in the positive experiments The second column gives the number of days between the first infected feed of the fly and its death and dissection :—

Table IV —Laboratory-bred Flies Positive Experiments

Expt.	Time, days	Proboscis		Proventriculus	Crop	Fore gut	Mid gut	Hind gut	Salivary glands
546	30	–		–	–	+	+	+	–
546	64	–		–	–	+ +	+ +	+ +	–
546	84	–		–	–	+ +	+ +	+ +	–
546	84	+ +		+ +	+ +	+ +	+ +	+ +	–
524	27	–		–	–	–	+ +	+ +	–
524	55	+		+	–	+ +	+ +	+ +	–
		Labial cavity.	Hypopharynx						
1787	27	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–
1787	28	+	–	+	–	+ +	+ +	+	–
1787	30	+ +	+ +	+ +	–	+ +	+ +	+ +	–
1858	17	+ +	+ +	+ +	–	+ +	+ +	+ +	–
1858	22	–	–	–	–	+	+ +	+	–
1858	23	+	–	+	–	+ +	+ +	+ +	–
1858	25	+ +	+ +	+	–	+ +	+ +	+ +	–
1858	26	+ +	+ +	+	–	+ +	+ +	+ +	–
1950	17	–	–	+	–	+ +	+ +	+ +	–
1950	19	+ +	+ +	–	–	+ +	+ +	+ +	–
1950	24	–	–	–	–	–	+	–	–
1950	26	+ +	+ +	+	–	+ +	+ +	+ +	–
1950	31	+ +	+ +	+	–	+ +	+ +	+ +	–
1950	31	+ +	+ +	+ +	–	+ +	+ +	+ +	–

In Experiments 546 and 524 there was no special examination of the hypopharynx, it is included in the general term "Proboscis" It was only

after the importance of the hypopharynx became evident that an examination of these separate parts of the proboscis was made

In Experiment 546 only one infective fly was found In Experiment 524 two infected flies were found, in one of these the development was incomplete, in the other complete In 1737 two flies were infective, in 1853 three, and in 1950 four In not a single fly was any invasion of the salivary glands noted.

The following Table gives the result of the dissection of the infected flies in the negative experiments —

Table V —Laboratory-bred Flies Negative Experiments

Expt	Time, days	Proboscis		Proventriculus	Crop	Fore-gut	Mid gut	Hind-gut	Salivary glands
		Labial cavity	Hypopharynx						
2115	9	–	–	–	–	–	+	–	–
2115	9	–	–	–	–	–	+ +	–	–
2115	9	+	–	+	–	+	+ +	+ +	–
2115	11	–	–	+	–	+	+ +	+	–

In the negative Experiment 1732 all the flies were found to be negative

In Experiment 2115 four infected flies were found, but in none of these had the development reached the hypopharynx, none of them were infective.

From a consideration of these tables it will be seen that *T. pecorum* belongs to the same group as *T simiæ* as regards its development in *G morsitans.* This development takes place at first in the intestine, then passes forward into the labial cavity, and finally invades the hypopharynx and there is completed

The Type of Trypanosomes Found in the Infected Flies

Plate 2 represents the developmental forms of *T. pecorum* in *G. morsitans.* In regard to the forms found in the intestine, it may be said that these are indistinguishable from the developmental forms of other pathogenic trypanosomes, and what was written in regard to *T simiæ** is equally applicable to *T pecorum.*

Figs 1 and 2 are forms from the proventriculus, and represent the dominant intestinal trypanosome forms passing forward to the labial cavity.

Figs. 3–8 represent early forms found in the labial cavity. These were seen adhering singly by their flagella to the labrum.

* 'Roy Soc. Proc.,' B, vol. 87, p. 65 (1913).

Trypanosoma pecorum
Development in *Glossina morsitans*

M. E. Bruce del.

X 2000

Figs. 9–11 are the ordinary forms found clinging by their flagellar ends to the labrum It will be seen that they have assumed the crithidial stage, a stage which seems to be a *sine quâ non* in the final stages of the cycle of development of all the pathogenic trypanosomes, and the interpretation of which is still obscure

Figs. 12–19 are various forms other than "blood forms" which have been squeezed out of the proboscis of a living infective fly. Fig 15 appears to be encysted.

Figs 20–29 are "blood forms" from the hypopharynx of dead infective flies, and also from living flies induced to salivate on a cover-glass They represent the final stage in the cycle of development and are the only infective forms.

Conclusions

1 That *T pecorum* is capable of passing through a cycle of development in *G morsitans*, the flies becoming infective some 20 days after feeding on an infected animal

2 That *T pecorum* belongs to the same group as *T simiæ*, the development taking place at first in the gut and afterwards passing forward into the labial cavity and finally into the hypopharynx.

3 That the final stage of the development only occurs in the hypopharynx, where the trypanosomes revert to the "blood form" and become capable of setting up infection if injected under the skin of healthy animals

DESCRIPTION OF PLATE

(See also pp 36 and 37 above)

Figs 1 and 2, trypanosome forms from proventriculus

Figs. 3–8, early infection of the labrum, the flagellates still retain the trypanosome characteristics.

Figs. 9–11, ordinary crithidial forms found adhering in masses to the labrum.

Figs 12–19, other forms from labial cavity

Figs 20–29 represent the final stage of the development in the hypopharynx—the infective or "blood form."

Stained Giemsa × 2000

Trypanosomes found in Wild Glossina morsitans *and Wild Game in the "Fly-Belt" of the Upper Shiré Valley*

By Surgeon-General Sir DAVID BRUCE, C.B., F.R.S., A.M.S., Major A. E. HAMERTON, D.S.O., and Captain D. P. WATSON, R.A.M.C., and Lady BRUCE, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912-14.)

(Received April 7,—Read June 18, 1914.)

INTRODUCTION

In June, 1913, one of the members of the Commission went to the Liwonde district to identify and isolate the various species of trypanosomes infecting the "fly" and wild game in the "fly-belt" which extends along the Upper Shiré River valley from Lake Pamalombe to the Murchison cataracts. This "fly-area" is, roughly speaking, 100 miles south of Kasu and the "Proclaimed Area." It is separated from the extensive "fly-area" of the plains on the west shore of Lake Nyasa, of which the "Proclaimed Area" forms a part, by a range of hills and high plateaux where the "fly" is absent, although there is nothing to prevent trypanosome-infected wild animals wandering from one "fly-belt" into the other.

The valley of the Upper Shiré is thickly populated, and the "fly-area" is crossed by two of the most frequented roads in Nyasaland, the grand trunk road running from Zomba to the north and the main road from Liwonde to Fort Johnston. Although thickly populated, human trypanosome disease, though probably existing, has not yet been discovered in this district.* The natives, however, can keep no cattle, and their goats and dogs are constantly destroyed by trypanosome diseases, so that they have to continually import these animals from the highlands.

Game is very abundant in this district, particularly in the dry season, when herds of eland, koodoo, waterbuck, and impala concentrate in the vicinity of the river. In the wet season elephant and buffalo wandering about the country frequently remain for many weeks in the impenetrable thickets and swampy "dambos" along the river banks. A characteristic feature of the flora of this district is the extensive forests of "sanya" trees, open forests of medium-sized trees, devoid of undergrowth, but carpeted with short wiry grass. Large herds of impala are always to be found in these forests, and tsetse flies are everywhere, being particularly numerous

* Since this was written cases of trypanosome disease in man have been found.

along the dusty tracks made by the antelope and around the pools where they drink

Nandumba's village (14° 40′ S lat., 35° 10′ E long), on the banks of the Shiré River, was selected as the locality for the camp in which to carry out experiments of feeding flies on healthy dogs and goats, monkeys being unobtainable. The experiments were carried out between the dates of June 19 and July 25, 1913

Methods Employed

The method employed in the feeding experiments was the same as described in a previous paper in the 'Proceedings,'* except that monkeys were unobtainable, and the flies were fed only twice on each animal.

All infected animals were subsequently taken to Kasu, the usual precautions being taken to prevent re-infection on the way, and the trypanosomes found in them were compared with the species and strains of trypanosomes obtained from human beings, various animals, and the flies in the Proclaimed Sleeping Sickness Area Special attention and study were devoted to the comparison of the strain of the trypanosome causing disease in man in Nyasaland—*Trypanosoma brucei vel rhodesiense*—from Nandumba's, with strains obtained from human beings, various animals, and the tsetse flies in the Proclaimed Area

The following Table gives in the first column the date the tsetse flies were first fed on the experimental animals, the second column the number of flies fed, and the signs plus and minus show the result of feeding the flies on the dog and goat •

Table I.—Infectivity of Wild *Glossina morsitans* in the Liwonde District

Date	No of flies fed	Dog				Goat			
		T brucei vel rhodesiense.	*T pecorum*	*T simiæ*	*T capræ.*	*T brucei vel rhodesiense*	*T pecorum*	*T simiæ*	*T capræ*
1913.									
June 19	78	+	—	—	—	—	+	+	+
„ 26	150	+	+	—	—	—	+	—	+
July 2	100	+	—	—	—	—	+	—	+
„ 4	450	+	—	—	—	—	+	—	+
„ 8	650	+	+	—	—	—	—	—	+
„ 15	650	+	—	—	—	—	+	—	—
„ 22	500	—	+	—	—	—	+	—	—

* 'Roy Soc. Soc.,' B, vol 86, pp 422 and 423 (1913)

It will be seen that the "fly" in the Upper Shiré district carries the same four species of trypanosomes as those found at Kasu in flies from the Proclaimed Sleeping Sickness Area *T. brucei vel rhodesiense, T pecorum, T simiæ*, and *T capræ*.

Here, in a series of seven experiments, all the animals on which the flies were fed developed trypanosome disease In six experiments the flies infected the dogs with *T brucei vel rhodesiense*, in the second and fifth there was a double infection with *T pecorum*, and in the seventh an infection with *T pecorum* alone None of the goats were infected with *T brucei* Six goats were infected with *T pecorum*, one with *T simiæ*, and five with *T capræ* It will be noticed that the smallest batch of flies used, a batch of 73, infected the dog with *T brucei*, and the goat with *T pecorum, T simiæ*, and *T capræ*

Examination of the Blood of Wild Animals in the Liwonde District

Whenever wild animals were killed their blood was examined for trypanosomes, which were identified by the microscope in stained films of the blood The following Table gives the results —

Table II

Animal	Species of trypanosomes found				
	T brucei vel rhodesiense	*T pecorum*	*T simiæ*	*T capræ*	*T ingens*
Zebra	–	–	–	–	–
Impala	–	–	–	–	–
"	–	–	–	–	–
"	–	–	–	+	–
"	–	–	–	–	–
"	–	+	–	–	–
"	–	+	–	–	–
"	–	–	–	–	–
Koodoo	–	–	–	–	–
"	–	–	–	+	–
Waterbuck	–	–	–	–	–
"	–	+	–	–	–
"	–	+	–	–	–
"	+	–	–	–	+
"	–	–	–	–	–
"	–	–	–	+	–

This Table, even in so small a series of animals examined, indicates that *T. pecorum* occurs frequently in the wild game, such as the impala and

waterbuck *T brucei vel rhodesiense* was found in only one animal out of the sixteen, *T simiæ* in none, and *T capræ* in three

Conclusions

1 The trypanosomes found in the wild *G morsitans* and wild game of the Upper Shiré "fly-area" are identical with those found 100 miles farther north in the Proclaimed Area

2 The trypanosome causing disease in man in Nyasaland—*T brucei vel rhodesiense*—is frequently met with, so that it is probable cases of this form of sleeping sickness will be found among the natives of this district

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The Food of Glossina morsitans.

By Surgeon-General Sir David Bruce, C B, F R S, A M S, Major A E Hamerton, D S O, and Captain D P Watson, R.A M C, and Lady Bruce, R R C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

(Received April 7,—Read June 18, 1914)

Five hundred flies, freshly caught in the Proclaimed Area, were killed by chloroform and the gut of each was roughly dissected out, smeared on a slide, fixed by osmic vapour and alcohol, and subsequently stained by Giemsa The flies were all caught in the bush, away from the paths, the fly-boys proceeding in single file and catching the flies with gauze nets as they circled round, or settled on the boys or the grass

The proportion of male flies to females caught was roughly two to one But only 30 females were used in the present experiment, the majority being sent to the breeding-station at Chunzi

Of the 500 flies examined, 288, or 57 6 per cent, were found to contain mammalian blood in a recognisable state No measurements were made of corpuscles, which in most cases were much altered by the digestive processes, but the small type of cell appeared to predominate, such as occurs in the hartebeeste, waterbuck, and other antelope.

In only three cases were nucleated red corpuscles found, and in two of these there was only a small proportion of nucleated blood mixed with a large amount of mammalian. In the third case the blood was all nucleated. Thus, of those flies which contained recognisable blood, only 1 0 per cent

contained nucleated blood. From measurements, it seems highly probable that in all three cases the blood was avian, not reptilian The average length of corpuscles and nuclei of blood from several different reptiles was measured and found to be—corpuscles 15 microns, nucleus 5 9 microns, while the blood of several different birds gave as the average—corpuscles 11 8 microns, nucleus 4 6 microns In the three cases under consideration the average of the corpuscles was 10 5, 10 0, and 10·0 microns respectively, and that of the nuclei 4 7, 4 8, and 4 4 microns Probably the size of the nucleus is the better guide than that of the whole corpuscle, as being less altered by digestion

In no case was vegetable matter noted in the intestinal contents

Trypanosomes were found in 14 flies—2 8 per cent—but many of the smears were so thick and so much obscured by the fat-body and other structures of the fly, that probably trypanosomes were present in other cases

Of the 30 female flies examined, 13, or 43 3 per cent, contained mammalian blood, and there was nothing to suggest that they differed in their feeding habits from the males

From experiments with flies in the laboratory, it was found that blood is recognisable in stained specimens for two to three days after a feed, but not beyond the third day Hence it may be inferred that, roughly, half the flies examined had fed within, at most, three days of their capture, and that therefore the flies feed naturally at least once every six days

Conclusions

1 The food of *Glossina morsitans* consists mainly of mammalian blood (99 per cent), chiefly from species of antelope, and what appeared to be avian blood (1 per cent).

2 There is no difference in the feeding habits of males and females.

3. Probably the flies feed once in five or six days

Infectivity of Glossina morsitans *in Nyasaland during* 1912 *and* 1913.

By Surgeon-General Sir David Bruce, C B, F R S, A M S, Major A E Hamerton, D.S O, and Captain D P Watson, R A M C, and Lady Bruce, R R C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

(Received April 7,—Read June 18, 1914)

Introduction

The object of this paper is to attempt to set up a rough standard of the proportion of infected to non-infected tsetse flies in an ordinary "fly-area" where wild game abounds It is thought that a standard of this kind may prove useful in the future

The flies were collected in the low country lying near the Commission's camp at Kasu, in what is known as the "Proclaimed" or Sleeping-Sickness Area of Nyasaland. This bit of country swarms with *Glossina morsitans* and wild game, the latter highly infected and well protected

In 1912 a total of 1975 flies were dissected between the months of January and November Of these 129 were found to be infected with trypanosomes—6 53 per cent Males, 86 per cent, females, 14 per cent

In 1913, 1060 flies were dissected, of which 91 were infected—8 58 per cent

The following Tables give the details :—

Table I —1912

No of fly	Proboscis	Proventriculus	Crop	Intestine	Salivary glands	Part of fly injected	Animal injected	Result
1	+ +	+ +	+ +	+ +				
2	–	–	–	+	–	Intestine	Dog	–
3	–	–	+	+ +		,,	,,	–
4	–			+	–	,,	Goat	–
5	–			+ +		,,	Dog	–
6	–			+ +		,,	Goat	–
7	–	+ +		+ +	–			
8	+ +			+ +		,,	Dog	–
9	+ +			–	–	Proboscis	Goat	–
10	+	+		+ +	–	,,	,,	–
11	–			+				
12	–			+	–			
13	–			+ +	–	Intestine	Dog	–
14	+ +			+ +	–			
15	–	–		+ +	–			
16	–			+ +	–			
17	+	–	–	–	–			
18	–			+ +	–			
19	+			–				

Table I —1912—*continued*

No of fly	Proboscis	Proventriculus	Crop	Intestine	Salivary glands	Part of fly injected	Animal injected	Result
20	−	−		+ +	−			
21				+				
22	+			+	−			
23	−			+	−			
24	+ +	+ +		+ +	−			
25	+ +			−				
26	−	−		+ +	−			
27	+ +	+ +	+	+ +	−			
28	+	−		+ +	−			
29	−			+ +	−	Intestine and salivary glands	Dog	−
30	−	−	−	+		Intestine	„	−
31	−			+	−			
32	+			+	−	„ Proboscis	„ „	− −
33	+ +			+				
34	−			+ +	−	Intestine	Goat	−
35	−			+ +		„	Dog	−
36	−			+ +	−	„	Goat	−
37	−	+ +		+ +	−	„ and salivary glands	„	−
38	−			+		Intestine	„	−
39	+			−		Proboscis	„	−
40	+			+		Intestine Proboscis	„ Dog	− −
41	−			+	−	Intestine	„	−
42	+			−		Proboscis	Goat	−
43	−			+ +		Intestine	„	−
44	+			−		Proboscis	„	−
45	−			+		Intestine	„	−
46	−			+		„	Dog	−
47	+			−		Proboscis	Goat	−
48	−			+ +		Intestine	Dog	−
49	+ +			+ +	−	„ Proboscis	„ „	− −
50	+			+		Intestine Proboscis	„ „	− −
51	−	−		+ +	−	Intestine	Goat	−
52	+			+		„	Dog	−
53	+			+		„ Proboscis	„ „	− −
54	−	−	−	+	−	Intestine	„	−
55	+			−		Proboscis	Goat	−
56	−			+	−	Intestine	„	−
57	−			+				
58	−			+ +	−	„	„	−
59	−			+				
60	−			+		„	„	−
61	+			−		„	„	−
62	+			+				
63	−	+ +		+	−			
64	+			−				
65	−			+	−			
66	+			+	−			
67	+			−				
68	+			−				
69	+			+ +	−			
70	−	−		+ +	−			
71	+			−	−			
72	−	+ +		+ +	−	Intestine	Monkey	−
73	+	+ +	+ +	+ +	−			

Table I.—1912—*continued*

No of fly	Proboscis	Proventriculus	Crop	Intestine	Salivary glands	Part of fly injected	Animal injected	Result
74	–			+ +				
75	–	–	+	+ +	–			
76	+			–				
77	+ +			+ +	–	Proboscis	Monkey	–
78	+			+				
79	+			+ +	–			
80	+			–				
81	–		–	+ +	–			
82	+			+ +	–			
83	+			–				
84	–	+ +		+ +	–			
85	–	–	–	+ +				
86	–		+	+	–			
87	+			–	–			
88	+			–				
89	–			+ +				
90	–			+	–			
91	–			+	–			
92	+			–				
93	–			+ +				
94	+ +	+ +	+ +	+ +	–			
95	–			+ +				
96	–	+		+	–			
97	+ +			–				
98				+	–			
99				+				
100	–			+	–			
101	–			+ +	–			
102	+ +			+ +	–			
103	–	+ +		+ +	–			
104		+ +	+ +	+ +	–			
105	–			+ +				
106	+			+ +				
107	+ +			+ +	–	Intestine	Dog	–
108	+ +			+ +	–			
109	+	+ +		+ +		,, and proboscis	Goat	+ *T simiae*
110	+ +	–		–	–	Proboscis	,,	–
111	+	+ +	+ +	+ +		,, and intestine	Dog	–
112	–			+ +	–	Intestine	,,	–
113	–			+ +	–	,,	,,	–
114	–	+ +		+ +				
115	–			+				
116	–	–		+	–			
117		–		+ +				
118	+	+ +	+ +	+ +	–	Intestine and salivary glands	,,	–
119	–			+ +		Intestine	,,	–
120	+			–				
121	+	–	–	+ +	–	,,	,,	–
122	–	+ +	–	+ +	–			
123	+		+	+ +	–	,,	,, and goat	–
124	+		+ +	+ +	–			–
125	–			+		Intestine	Dog	–
126	–	–	–	+ +	–	,,	Goat	–
127	+		+	+	–			
128	–	+	–	+ +	+ +	Salivary glands	Rat	+ *T brucei*
129	–	+	–	+ +	+ +	,,	,,	+ *T brucei*

It will be seen from the above table that 60 attempts to determine the infectivity of the flies were made by injecting emulsions of the infected organs into healthy animals In only three cases did the animals become infected once with *Trypanosoma simiæ* and twice with *T. brucei vel rhodesiense* The usual experiment was to inject the contents of the intestine into dogs or goats, which is known now to be useless, as the developmental forms in the intestine are not infective Doubtless more positive results could be got at present with more knowledge of the laws which govern infectivity. Only in two cases were the salivary glands found to be invaded. This infection, of course, could only be *T brucei vel rhodesiense*, and this was confirmed by injecting the glands into rats

In 1912 no attempt was made to diagnose directly the species of trypanosomes with which the flies were infected, but in 1913 this was done, as by that time a good deal of experience had been gained For example, invasion of the salivary glands could only be *T brucei vel rhodesiense*, invasion of the intestine, labial cavity and hypopharynx meant *T pecorum* or *T. simiæ*, and size would distinguish between the two Lastly, if only the labial cavity and hypopharynx were seen to contain flagellates, then *T capræ* was indicated, and here also the size and character of the trypanosomes in the hypopharynx would assist in the diagnosis

Table II —1913

No of fly	Proboscis		Intestine	Salivary glands	Species of trypanosome
	Labial cavity.	Hypopharynx			
1	+		+		*T pecorum*
2	+		−		*T capræ*
3	+		−		,,
4	+		−		,,
5	+		+		*T simiæ*
6	+		−	−	*T capræ*
7	−	+	+	−	
8	−	−	+		
9	+		+		*T simiæ*
10	+		−		*T capræ*
11	+		−		,,
12	−		+ +	−	
13	−		+	−	
14	−		+	−	
15	+		+ +		*T simiæ*
16	+		+		,,
17	+				*T capræ*
18	+	+	+	−	*T pecorum.*
19	+		+		
20	+		+	−	
21	−	−	+ +	+	*T. brucei*
22	−		+	−	
23	+		−		*T capræ*

Table II.—1913—*continued.*

No of fly.	Proboscis		Intestine	Salivary glands	Species of trypanosome
	Labial cavity	Hypopharynx			
24	+		−		*T capræ*
25	−		+		
26	−		+	−	
27	+	+	+	−	
28	−		+	−	
29	−		+	−	
30	−		+	−	
31	+		+	−	*T pecorum*
32	+		+		*T simiæ*
33	−		+		
34	−		+		
35	−		+	−	
36	+		+		,,
37	−		+	−	
38	+		+	−	*T pecorum*
39	+		+	−	*T simiæ*
40	+		+	−	,,
41	+		−	−	*T capræ*
42	−		+	−	
43	−		+	−	
44	+		−		,,
45	−		+	−	
46	−		+	−	
47	+		−	−	,,
48	−		+	−	
49	−		+	−	
50	−		+	−	
51	−		+	−	
52	+	−	+	−	
53	+		−		
54	+		−		
55	−		+		
56	+	+	+		
57	+	−	−	−	
58	+	−	−	−	
59	−	−	+	−	
60	+	+	+	−	*T simiæ*
61	+		+		,,
62	−		+	−	
63	+		−		*T capræ*
64	+	+	+		*T pecorum*
65	−		+	−	
66	+	+	−		*T capræ*
67	−	−	+	−	
68	+	−	−	−	
69	+	−	−	−	
70	−	−	+	−	
71	+	−	−		
72	+	−	−		
73	−	−	+		
74	+	−			
75	+	−			
76	+	−	+	−	
77	+	−	−	−	
78	+	−	−	−	
79	−	+	+	−	
80	+		+		*T simiæ*
81	−		+	−	

Table II.—1913—*continued*

No of fly	Proboscis		Intestine	Salivary glands	Species of trypanosome
	Labial cavity	Hypopharynx			
82	+	–	–	–	
83	+		+		*T pecorum*
84	+		+		*T simiæ*
85	–	–	+	–	
86	+	+	–	–	
87	+	–	–	–	
88	+	–	–	–	
89	+	–	+	–	
90	–	–	+	–	
91	–	–	+	–	

In 1913 no injections of the contents of organs were made into healthy animals. The direct diagnosis of the species of trypanosomes by examination of the fly took the place of inoculation

From the above table it will be seen that in 1060 flies *T brucei vel rhodesiense* was found once, *T pecorum* six times, *T simiæ* 12 times, and *T capræ* 14 times It must, however, be confessed that the margin of error in this calculation may be large

CONCLUSION

In 1912, 6 53 per cent of the *G morsitans* found in the "Proclaimed" or Sleeping-Sickness Area, Nyasaland, were infected with pathogenic trypanosomes, in 1913, 8 58 per cent

The Various Inclinations of the Electrical Axis of the Human Heart. Part IA.—*The Normal Heart Effects of Respiration.**

By Augustus D. Waller, M.D., F.R.S.

(Received March 30,—Read May 14, 1914.)

(Plates 3–6.)

In my first account of the electrical action of the human heart,† I made no allusion to the influence upon the electrical pulse of the movements of respiration. I noticed that influence indeed which is especially well marked in my own case (where the heart happens to be of the complete horizontal type) but only as disturbing the demonstration, and in some cases rendering the direction of the pulse uncertain. I noticed in particular that when demonstrating the transverse effect from the two hands, best effects were shown by holding my breath in expiration, and that these effects were markedly diminished during deep inspiration. I imagined at that time that the effect was due to a disfavouring of current spread from the heart by reason of the distended lung, but was puzzled by the fact that with the axial lead (right hand and left foot) the electrical pulse was augmented during inspiration instead of diminished as was the case with the transverse lead. I did not, however, follow up the clue afforded by this discrepancy, and it was only much later, *i.e.* after the introduction by Einthoven of the string-galvanometer and the observations of Einthoven, Kraus and Nicolai, Samojloff and others, that the meaning of the discrepancy and with it the whole mechanism of the respiratory effects became clear. The variations of amplitude are, if not entirely, almost entirely due to the rise and fall of the diaphragm, raising and lowering the heart as a lever hinged at the aortic end and thus widening and narrowing the "axial angle." (By axial angle I mean the angle formed with the vertical by the current axis of the heart or line of greatest potential difference at right angles to the equator OO.)

In 1889 I represented this angle as being 45° to the left (and 45° to the right in cases of *situs viscerum inversus*), and drew two series of curved

* The method followed was the same as that followed for the observations of Part I. A Bock-Thoma galvanometer was used for these observations, the deflections being standardised whenever necessary by a millivolt switched into the circuit. For the careful comparison of values in different leads or between the values obtained at different times, a standard millivolt deflection was of course taken, and the proportional correction applied, if necessary. But in comparisons taken between the two sides of the body the standard deflection was found to be so invariable that it was often omitted.

† "On the Electromotive Changes connected with the Beat of the Mammalian Heart, and of the Human Heart in particular," 'Phil. Trans.,' B, vol. 80, p. 169 (1889).

equipotential lines at opposite ends of the heart. To-day I represent the angle as 30° to the left (it can range in healthy subjects between 10° to the right and 100° to the left), and draw straight equipotential lines, parallel with the equator, at right angles to the current-axis CC (fig 1). To-day as in 1889 I regard as essential the distinction between "strong" leads (left superior

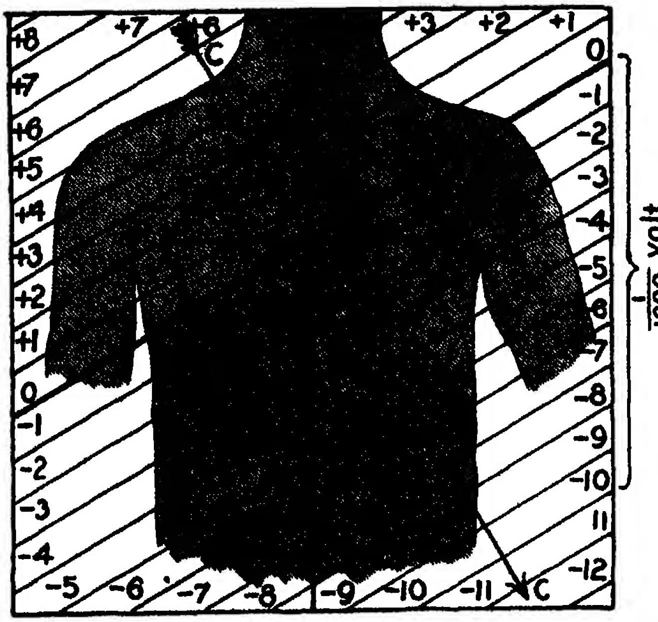

FIG 1—A typical heart with an oblique electrical axis $a = 30°$

OO is the equator, CC the current-axis Equipotential lines drawn parallel to the equator, and at right angles to the current-axis at regular intervals of 1/10000th volt a indicates the "axial angle" formed with the vertical MF by the current-axis CC $\tan a = 2\frac{R-L}{R+L}$ where R and L are the electromotive values of the Right and Left inferior ventricular spikes

ML, and right inferior RF) and "weak" leads (right superior MR, and left inferior LF) (see figs) The present diagram (fig 1) is drawn so as to exhibit the relative values of potential difference between the led-off points R, M, L, F, by the number of equipotential lines which they include. Each unit $= 1 \times 10^{-4}$ volt. Thus *e g* in fig 1 it is to be seen that (approximately) $MR = 1.5$, $ML = 5.5$, $RL = 4.0$, $RF = 9.0$, $LF = 5.0$ ten-thousandths of a volt

At pages 518–9 of the communication to which the present paper belongs* it is stated that a full consideration of the effects of respiration upon the (amplitude of the) electrocardiogram is a necessary preliminary to the due understanding of the physiological and pathological departures from the

* 'Roy. Soc. Proc.,' B, vol 86 (1913).

normal type. The consideration of the effects of respiration appeared to me at that time as a side-issue to be cleared up by a few carefully planned observations, whereas it now presents itself as an extremely simple exercise in elementary trigonometry on the main line of the principal argument

The essential distinction between favourable or strong leads (left superior and right inferior) and unfavourable or "weak" leads (right superior and left inferior) which was the principal result of my first investigation of the subject, afford, when their data are converted into simple ratios, sinusoidal curves which plotted upon millimetre paper exhibit directly the quantitative relations between the electrical effects of the heart, whether horizontal or vertical (as described in Part I) or at various inclinations in accordance with respiratory alterations of the cardiac axis

The facts will be best presented by a detailed account of two typical cases

The Case of B O B—*An Oblique Heart.*

Fig 2 (Plate 3) gives the transverse, right inferior and left inferior records taken simultaneously with the record of respiration slow and deep so as to emphasise the effects upon the heart, which in this subject had been determined as having an electrical axis forming an angle not exceeding 30° with the vertical during quiet breathing

The amplitude of the spike in the *transverse* record varies between a maximum of 20 mm in expiration and a minimum of 15 mm in inspiration The waxing and waning is very regular and at once suggests a sinusoidal curve

In the *right inferior* record the respiratory variation of amplitude is much less pronounced and regular within a range that may be taken as 27 5 mm. in expiration and 24 mm in inspiration.

In the *left inferior* record the variation is regular and large and suggestive of a sinusoidal curve The range is measured as between the values = 6 mm immediately after the culmination of expiration, and = 14 mm during inspiration (In these three records the deflection by 1/1000 volt through body and galvanometer was 18 mm.)

From these values right and left below the heart we calculate that —

$$\text{The expiratory } \tan\alpha = 2\frac{27{\cdot}5-6}{27\,5+6} = 1\,28, \qquad \alpha = 52^\circ$$

$$\text{The inspiratory } \tan\alpha = 2\frac{24-14}{24+14} = 0\,53, \qquad \alpha = 28^\circ.$$

$$\text{Diff} = 24^\circ.$$

In the *right superior* record the respiratory variation is small and fairly regular between values that may on the average be taken as = 5 mm. in expiration and = 7·5 mm in inspiration.

In the *left superior* record the variation is large and fairly regular between values = 26 mm. in expiration and = 18 mm in inspiration.

The right and left measurements give for the superior angle α above the heart the following values :—

In expiration—

$$\tan\alpha = \frac{26-5}{26+5} = \frac{21}{31} = 0{\cdot}68, \quad \therefore \quad \alpha = 34^\circ.$$

In inspiration—

$$\tan\alpha = \frac{18-7{\cdot}5}{18+7{\cdot}5} = \frac{10{\cdot}5}{25{\cdot}5} = 0{\cdot}41, \quad \therefore \quad \alpha = 22^\circ.$$

$$\text{Diff} = \overline{12^\circ}$$

From these results we see that in the case of an oblique heart the effect of inspiration is to weaken the strong leads and to strengthen the weak leads But this rule, while affording a useful mnemonic, is applicable only to oblique and vertical hearts * The inspiratory diminution of the superior angle has in this case come out as 12°, as compared with 24° for the inferior angle. This is as might be expected from the fact that the basal attachment of the heart cannot be rotated to the same extent as its apical free portion Obviously we cannot expect that the angle calculated above the heart from the three points M, R, L, should necessarily be identical with that calculated below the heart from the three points F, R, L

(The records of this case incidentally afford a striking example of the variations of pulse-frequency that are associated with the two phases of respiration, sometimes in the human subject, always in the dog I have discussed them elsewhere under the title "Dog Pulse"† Their consideration does not belong to this portion of the subject)

The Case of A. D W.—*A Heart of the Horizontal Type*

Fig 3 gives the transverse, right inferior, and left inferior records taken simultaneously with a record of respiration, slow and deep so as to emphasise the effects upon the heart, which in this subject had been determined as having an approximately horizontal electrical axis The spikes of the weak leads (right superior and left inferior) are accordingly reversed, as was explained in Part I.

In the *transverse* records the respiratory variations of the spike are regular and large, between values = 15 mm in expiration and = 10 mm. in inspiration It may be noticed in this record that inspiratory diminution goes on

* I speak of hearts as "vertical," "oblique," and "horizontal," according as the axial angle α is between 0° and 30°, 30° and 60°, 60° and 90° The contrasted types are vertical and horizontal, oblique hearts belong to the vertical type

† 'Physiol. Soc. Proc.,' June 28, 1913

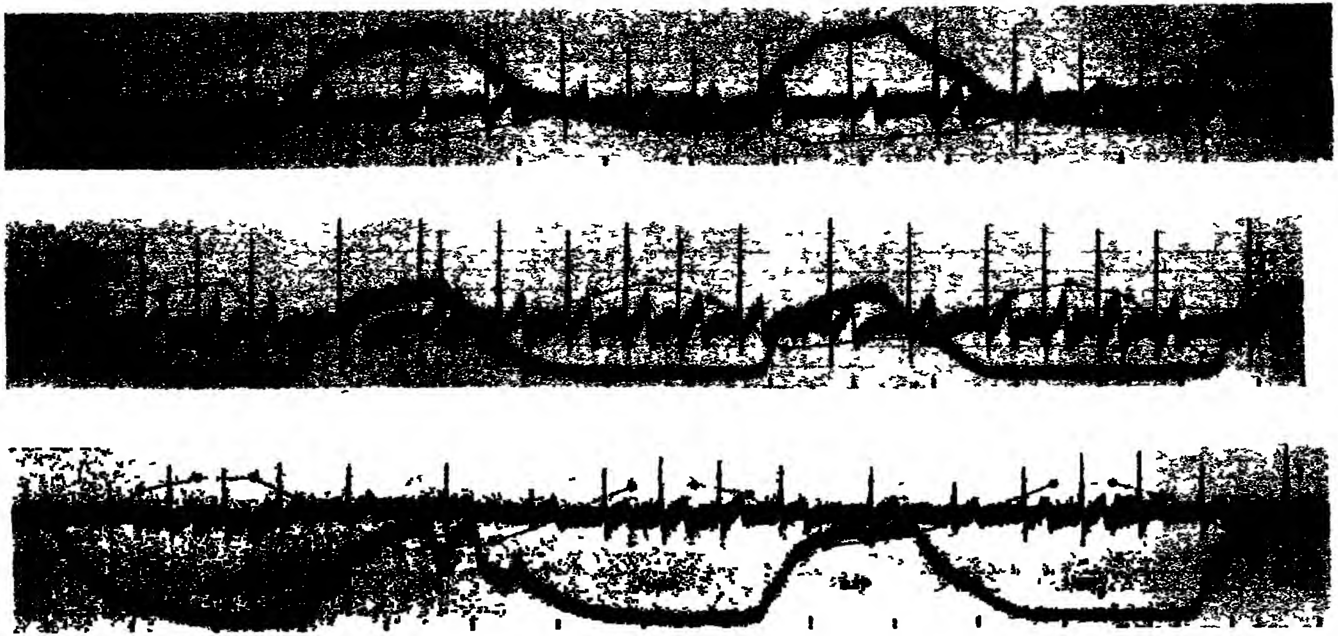

Fig 2 — The Case of B O B An oblique heart Effect of respiration upon the electrocardiogram (and upon the pulse-frequency) in the *transverse*, *right inferior* and *left inferior* leads

In the *transverse* lead (upper line) the ventricular spike V_1 is distinctly diminished during inspiration

In the *right inferior* lead (middle line) it is on the whole diminished during inspiration

In the *left inferior* lead (lower line) it is distinctly increased during inspiration

The pulse-frequency in this case (as shown by the thin line between dots) is greater during inspiration than during expiration.

The phases of respiration are recorded simultaneously with the electrocardiogram

FIG 3 - THE CASE OF A D W A horizontal heart Effect of respiration upon the electrocardiogram (and upon the pulse frequency) in the transverse, right inferior and left inferior leads

In the *transverse* lead (upper line) the ventricular spike is distinctly diminished during inspiration

In the *right inferior* lead (middle line) it is increased during inspiration

In the *left inferior* lead (lower line) the ventricular spike is negative, and thus negative spike is diminished during inspiration

The pulse-frequency (indicated only in the transverse record) is slightly increased during inspiration

into the beginning of expiration, and that the expiratory augmentation goes on into the beginning of inspiration This lag is probably mechanical, the pneumograph begins to indicate sooner than the heart begins to be raised and lowered. The effects are similar to those seen with the vertical heart, viz., inspiratory diminution, expiratory augmentation.

In the *right inferior* record the respiratory variations of amplitude are regular and relatively large, *i e* in relation to the absolute magnitude of the spike, which in the horizontal heart is small The range is approximately between 6 mm. in expiration and 9 mm in inspiration

The *left inferior* record exhibits the negative spike characteristic of the horizontal or soft heart, and with respiration well marked augmentation during expiration and diminution during inspiration. The values as read on this record reach to -13 mm at the end of expiration and to -6 mm. at the end of inspiration.

The angles as calculated from these right- and left-hand values are :—

In expiration—

$$\tan\alpha = 2\frac{9+13}{9-13} = \frac{44}{-4} = -11, \quad \therefore \alpha = 95^\circ$$

In inspiration—

$$\tan\alpha = 2\frac{6-6}{6+6} = 0, \quad \therefore \alpha = 90^\circ.$$

The values for the *right* and *left superior* leads of this subject (of which tracings are not reproduced here) were as follows —

	Right superior	Left superior	tan a.	a
	mm	mm		°
In expiration	−5·0	7 5	5·0	79
In inspiration	−2 5	10 0	1 67	59

Reviewing the results of this case of horizontal heart as to the general effects of respiration in comparison with the results obtained for the oblique or vertical heart, it appears that these effects with inspiration are as follows :—

	B O B Vertical (and oblique)	A D W Horizontal
Transverse spike .	Decrease from 20·0 to 15·0 mm.	Decrease from 15·0 to 10·0 mm
Right inferior spike	" " 27 5 " 24·0 "	Increase " 6·0 " 9·0 "
Left " "	Increase " 6·0 " 14 0 "	Decrease " −18·0 " −6·0 "
Right superior "	" " 5·0 " 7 5 "	" " − 5 0 " −2 5 "
Left " "	Decrease " 26·0 " 18·0 "	Increase " 7 5 " 10 5 "

There is at first sight a flagrant discrepancy between the effects of respiration upon the two types of heart. The only case in which the effect is similar in both is that of the transverse lead The provisional rule framed above for the vertical heart — inspiratory weakening of strong leads and strengthening of weak leads — is evidently inapplicable to the horizontal heart The cases of the right superior and left inferior (decreased negative spike) are indeed amenable to it, since decreased negative as well as increased positive amount to electrical strengthening. But the cases of the left superior and right inferior, where after some uncertainty it became clear that a positive spike is increased for the horizontal, decreased for the vertical heart, are in apparent contradiction of the rule

Graphically expressed the angles in the two subjects B O B and A. D. W in inspiration and in expiration are as follows :—

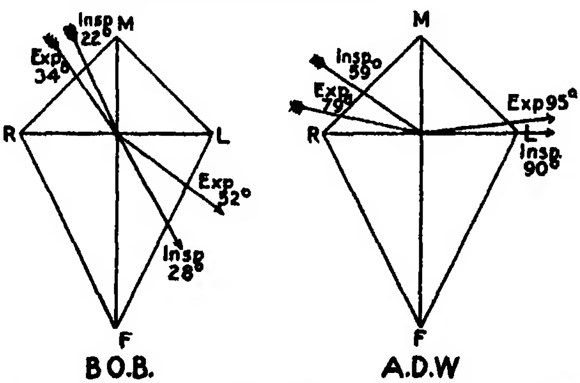

FIG 4 — Diagrams to indicate the value of the axial angle a in inspiration and in expiration of the subjects B O B and A D W, calculated from the electromotive values of the right and left superior spikes and of the right and left inferior spikes

	B O B		A D W	
	tan a	a	tan a	a
		°		°
Supr insp	$\frac{18-7\ 5}{18+7\ 5} = \frac{10\ 5}{25\ 5} = 0\ 41$	22	$\frac{10+2\ 5}{10-2\ 5} = \frac{12\ 5}{7\ 5} = 1{\cdot}67$	59
Supr exp	$\frac{26-5}{26+5} = \frac{21}{31} = 0\ 68$	34	$\frac{7\ 5+5}{7\ 5-5} = \frac{12\ 5}{2\ 5} = 5$	79
Inf insp	$2\frac{24-14}{24+14} = \frac{20}{38} = 0\ 53$	28	$2\frac{6-6}{6+6} = 0$	90
Inf exp	$2\frac{27{\cdot}5-6}{27\ 5+6} = \frac{43}{33\ 5} = 1\ 28$	52	$2\frac{9+13}{9-13} = \frac{44}{-4} = -11$	95

But the general relations between the varying "strengths" of leads in different hearts and in different phases of respiration will become most clear

from a consideration of the simple trigonometrical ratios of a varying axial angle

The transverse lead is the simplest to consider, and its simplest case is that of the horizontal heart in which the axial angle during quiet respiration is 90° In this position, regarding the heart as forming (electrically) a horizontal lever, it is evident that with this type of heart the transverse electrical effect has its maximum value, and that the effect must be diminished by either rise or fall of the diaphragm, the diminution will be proportional to the sine of the altered axial angle Taking as unity or 10 this maximal value with the chest at rest, we shall have the altered values = 9 8, 9 4, 8 7, 7 7, 6 4, 5 0, if with fall of the diaphragm the angle 90° is changed to 80°, 70°, 60°, 50°, 40°, 30°

Thus the theoretical alterations of magnitude in the transverse lead with alterations of the axial angle must be in ratio with the numerical values of the sines of the altered angle And as the reference curve to which to compare our observations we have the simple curve of sines —

	0	1736	3420	5000	6428	7660	8660	9397	9848	10000
at	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°

The superior leads, right and left, come next in order of simplicity The former (mouth and right hand), as stated in 1889, is "weak," being most nearly in accordance of direction with the direction of the equator, the latter is "strong," being most nearly in accordance of direction with the direction of the current-axis The angle RML (fig 1) is taken as being = 90° In the case of the horizontal heart with the axial angle = 90° it is evident that the electrical effects along MR, ML, are equal and opposite, with values $= \pm$ RL$(\cos 45°)^2 = \pm 50$. With the axial angle altered $\pm 10°$ by respiration the effects become —

$$\text{Along MR} = 100 \cos(45° + 10°) \times \cos 45° = 40,$$

and

$$\text{along ML} = 100 \cos(45° - 10°) \times \cos 45° = 58$$

With a perfectly vertical heart, *i e* with an axial angle = 0°, the effects are again equal, viz, $100(\cos 45°)^2 = 50$

The theoretical alterations of magnitude of the superior leads with alterations of the axial angle must be in numerical ratio with the cosines of the altered angle. We have as the reference curve to which to compare our observations, a simple sine curve formed by the tabular values of cosines multiplied by a constant factor to correct for projection between RL and the two sides MR, ML—in this case multiplied by cos 45°. The electromotive values for the superior leads for values of the axial angle from 0° to 90° are thus.—

°	Left superior $\cos 45° \times \cos (45° - \alpha)$	Right superior $\cos 45° \times \cos (45° + \alpha)$
0	5000	5000
10	5793	4056
20	6409	2988
30	6829	1830
40	7014	0616
45	7071	0000
50	7014	−0616
60	6829	−1830
70	6409	−2988
80	5793	−4056
90	5000	−5000

It will be noticed that the right-hand values steadily diminish as the axial angle increases from 0° to 90° from their maximum of 50 to their minimum of −50, passing through the value 0 when $\alpha = 45$, that the left-hand values between $\alpha = 0°$ and $\alpha = 90°$ increase from 50 to a maximum of 71 (7071) at 45° and then decrease again to 50. The right-hand lead is "weak," the left-hand lead is "strong." In the strong lead the first ventricular wave V_I is positive in both types of heart—vertical and horizontal. It is increased by inspiration in the horizontal heart, decreased by inspiration in the vertical heart.

In the formulæ given above, for the calculation of the numerical values of right and left superior leads, we have taken the vertical angle at $M = 90°$, so that the semi-vertical angle $M/2 = 45°$ [and that the formula for calculating α from known values of R and L is $\tan \alpha = (L-R)/(L+R)$]. Generalising for any value of M the formulæ for the superior leads become.—

For left-hand values— $\cos \frac{M}{2} \times \cos\left(\frac{M}{2} - \alpha\right)$,

For right-hand values— $\cos \frac{M}{2} \times \cos\left(\frac{M}{2} + \alpha\right)$,

and for calculation of α from known values of R and L,

$$\tan \alpha = \cot \frac{M}{2} \cdot \frac{L-R}{L+R}.$$

The right and left inferior leads (right hand and either foot, left hand and either foot) are at first sight somewhat less simple, but they are readily simplified As was shown in my first observations of 1889, the two feet are practically isoelectric, and we may therefore regard as being electrically identical the two right inferior leads (axial and right lateral) and the two left inferior leads (equatorial and left lateral). The two feet

are thus taken to be represented by a single point F The right-hand lead RF, being most parallel to the normally oblique current-axis, is the strong lead, the left-hand lead, being least parallel to the current-axis, is the weak lead.

The general formulæ for the inferior leads are —

For right-hand values— $\frac{1}{\cos F/2} \times \cos(F/2-\alpha)$,

For left-hand values— $\frac{1}{\cos F/2} \times \cos(F/2+\alpha)$,

and for calculation of α from known values of R and L

$$\tan\alpha = \cot\frac{F}{2} \times \frac{R-L}{R+L}$$

In Part I the angle F has been taken as 53°, so that $F/2 = 26.5°$ and $\tan\alpha = 2\frac{R-L}{R+L}$ The values of R and L at different values of α are now—

For the right side— $\frac{1}{\cos 26.5°} \times \cos(26.5°-\alpha)$,

For the left side— $\frac{1}{\cos 26.5°} \times \cos(26.5°+\alpha)$

The results come out as follows —

	Right inferior $\cos(26.5°-\alpha)/\cos 26.5°$	Left inferior $\cos(26.5°+\alpha)/\cos 26.5°$
°		
0	1000	1000
10	1071	898
20	1110	769
30	1115	617
40	1086	445
50	1024	261
60	932	68
70	811	−126
80	665	−317
90	500	−500

Similarly, we may work out the values of the leads which have been assumed above as identical, *i e* right lateral and axial, left lateral and equatorial. But it would be tedious and unnecessary to give this in detail, since, as will presently be seen, the results are most easily and quickly obtained by a geometrical model, which gives immediately the results that have been considered up to this point. This model is intended also to render evident the meaning of the apparent discrepancies between vertical and horizontal hearts as regards the effects of respiration, and to give

geometrically the theoretical values of the first ventricular spike V_I in all leads at all values of the axial angle α

The quadrilateral figure (fig 5) RMLF, in which CM = ½(LR) and LR = CF, so that cot ½ the vertical angle at M = 1, and cot ½ the vertical angle at F = 2, represents at its points the leading-off points mouth, hands, and feet. It is pinned by its centre to the centre of a field ruled in parallel (equipotential) lines, each division in this figure represents 1×10^{-4} volt, so that 10 divisions are equivalent to 1 millivolt. Lines at right angles to the equipotential lines (not drawn) would represent lines of force, the arrow CC through the centre of the field represents the current-axis, the line OO at right angles to CC represents the equator

The figure and the field can be rotated in relation to each other round the centre C, so that the current-axis CC is placed at any given angle from the vertical The figure being weighted so as to remain vertical, the field when tilted gives any desired inclination of the current axis, and the points R, M, L, F, occupy positions upon the field that indicate directly the potential differences between them, *i e.*, in the different leads Thus, *e g*, if the current-axis is inclined 30° the values in relation to O of the four points R, M, L, F, will be respectively + 2 5, + 4 7, − 2 5 and − 8 6 above and below zero, and the potential differences in the various leads will be given by the differences of level between various pairs of points, as follows —

Current-axis = 30° with vertical (as in fig 1)

MR (right superior)	43 − 25 = 18	$\times 10^{-5}$ volt
ML (left superior)	43 + 25 = 68	„
RL (transverse) ..	25 + 25 = 50	„
RF (right inferior)	86 + 25 = 111	„
LF (left inferior) .	86 − 25 = 61	„
MF (longitudinal) .	86 + 43 = 129	„

Obviously as regards the two sides of the body, MR and LF are relatively "weak" leads, ML and RF are relatively "strong" leads Similarly the potential difference in the several leads can be determined for any value of α by pricking off the positions of the points M, R, L, F, and measuring off the differences of level between pairs of points. It will be realised at once that with high values of α the direction of the weak leads is reversed—*e g* at 80° the right superior potential difference is seen to be −40 and the left inferior potential difference −32. At 90° both weak leads = −50 and both the strong leads = +50

Plotted out upon squared paper the values thus obtained give the curves represented in fig 6, which are obviously sinusoidal curves, most obviously

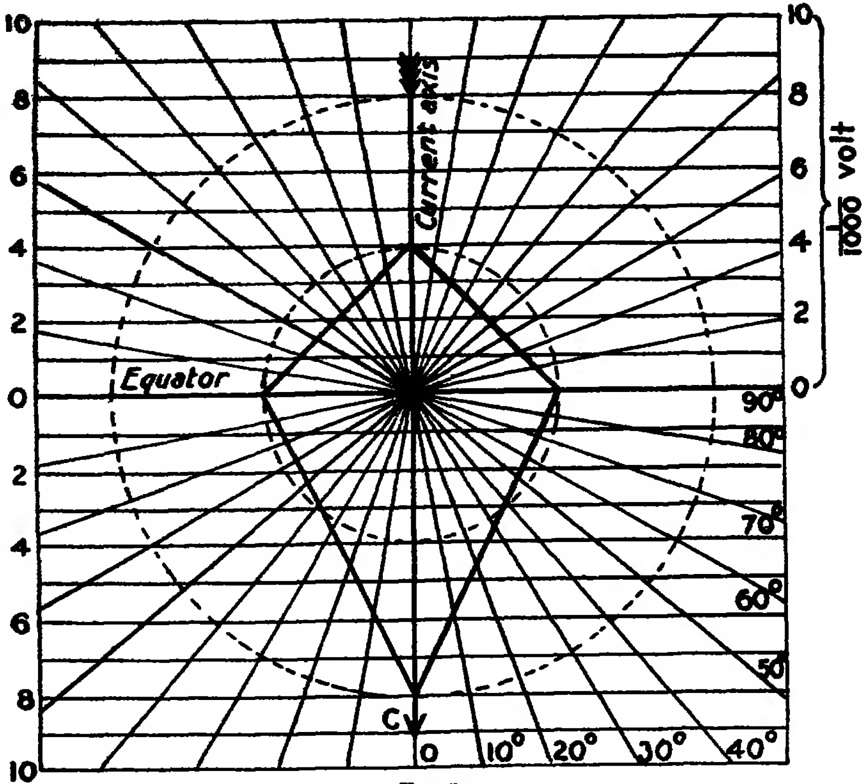

Fig 5

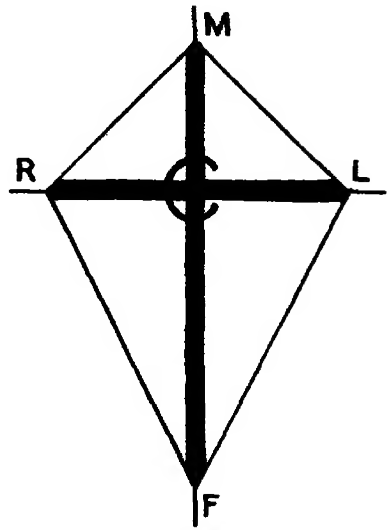

Fig 5A

Figs. 5 and 5A —Diagram-model for the automatic calculation of the electromotive values of the ventricular spike V, at varying values of the axial angle a, as described in the text

The fig. 5A should be cut off and superposed upon fig 5 by a pin through the centre of the two figures When rotated round this centre the position of the points M, R, L, F, of fig 5A can be pricked off upon the background of equipotential lines which represent steps of 1/10000th volt

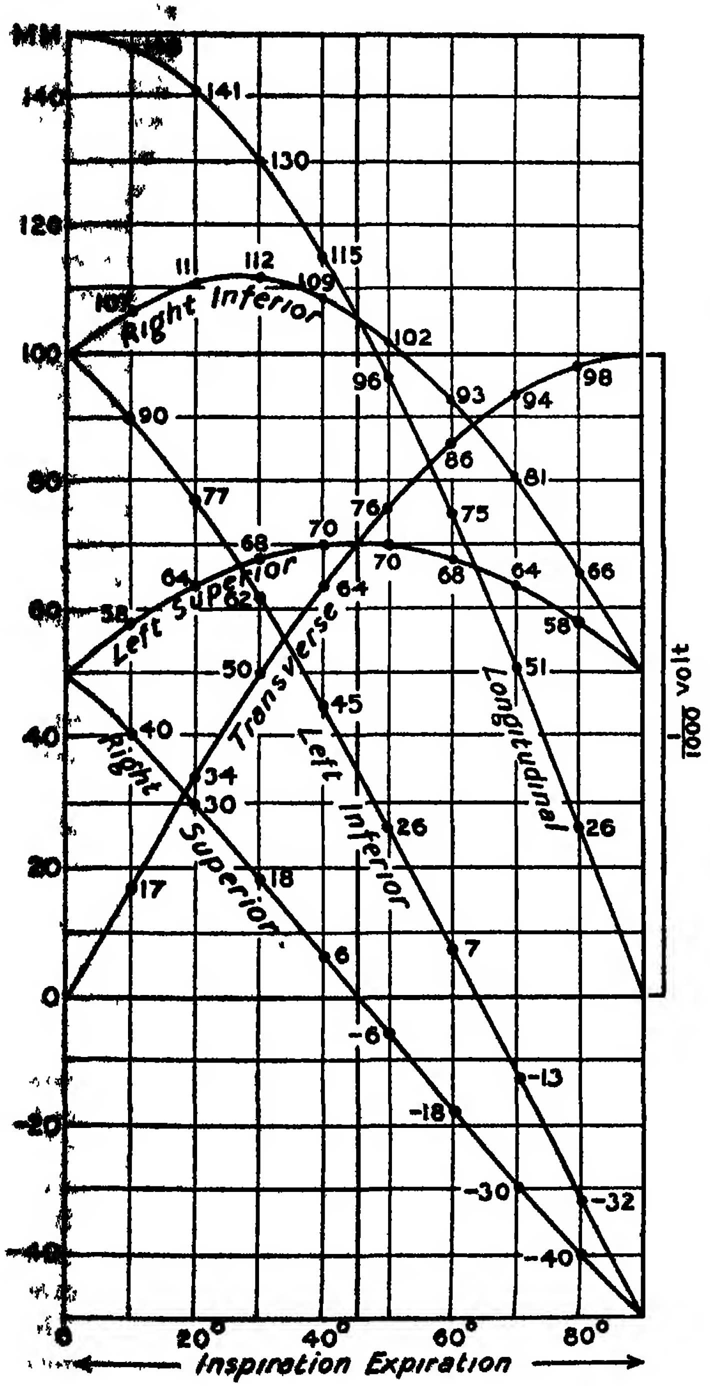

FIG 6 —Curves giving the electromotive values of the several heart leads with values of the axial angle *a* from 0° to 90°

The value of, *e g*, the transverse lead increases with increase of *a*, it decreases with inspiratory decrease of *a*. The value of, *e g* the left inferior lead decreases with increase of *a* and becomes negative when *a* is above 64° The negative left inferior spike of a horizontal heart is diminished by a deep inspiration and can sometimes be reversed to positive The positive left inferior spike of an oblique heart is increased by inspiration and can sometimes be reversed to negative by a maximum effort of expiration See figs 7 and 9.

so for the transverse values, which can be directly read off from a sine table, for the other leads the curves can be verified by working out their appropriate formulæ. Fig 6 is useful as showing at a glance the theoretical values to which the observed values must approximate in the several leads with hearts of various inclinations, and as regards the present paper affords the readiest mode of explanation of the otherwise somewhat perplexing effects of respiration upon the amplitude of the electro-cardiogram. Inspiration by reason of descent of the diaphragm and elevation of the ribs causes a clockwise rotation of the electrical axis of the heart, *i e* a diminution of the angle α above and below the heart. Reading the appropriate line on fig 6 from right to left, *i e* in the direction of inspiratory decrease of α, we see by its rise or fall whether and how much increase or decrease of the ventricular spike is to be anticipated Thus the transverse must be decreased with inspiration (or increased with expiration); the longitudinal, the right superior, and the left inferior increased And as regards the troublesome case of the two strong leads we realise at a glance how it happens that at high values of α, *i e.* with a "horizontal" axis, we find inspiratory increase of the strong leads, whereas at low values of α, *i.e* with an axis at less than 30°, we find inspiratory decrease.

This figure also supplies an explanation of the following two experiments, one or other of which can nearly always be repeated with success upon a heart of which the electrical axis happens to be of suitable inclination —

Experiment 1 —With an approximately horizontal heart the normally negative left inferior spike may be abolished and rendered positive during maximal inspiration An example of this experiment is given in fig 7.

Experiment 2.—With an oblique heart ($\alpha = 30°$ to $45°$) the normally positive left inferior spike may be abolished and rendered negative during maximal expiration. An example of this experiment is given in fig. 8.

[*Note* —It is not always easy to decide what are the actual measurements to be taken for calculation In simple cases, *i.e.*, in cases of vertical heart where V_I is large and positive on both sides, there is no difficulty in determining these values. Nor is there any serious ambiguity for the case of the clearly horizontal heart where V_I is large and at once negative. But the not infrequent cases where V_I is double, composed of a small positive followed by a large negative, or of two small positives separated by a negative movement, cannot be dealt with with the same degree of certainty. The indicator does not take up a decided position, the current axis resultant from opposed and nearly balanced forces fluctuates to and fro, and the obvious

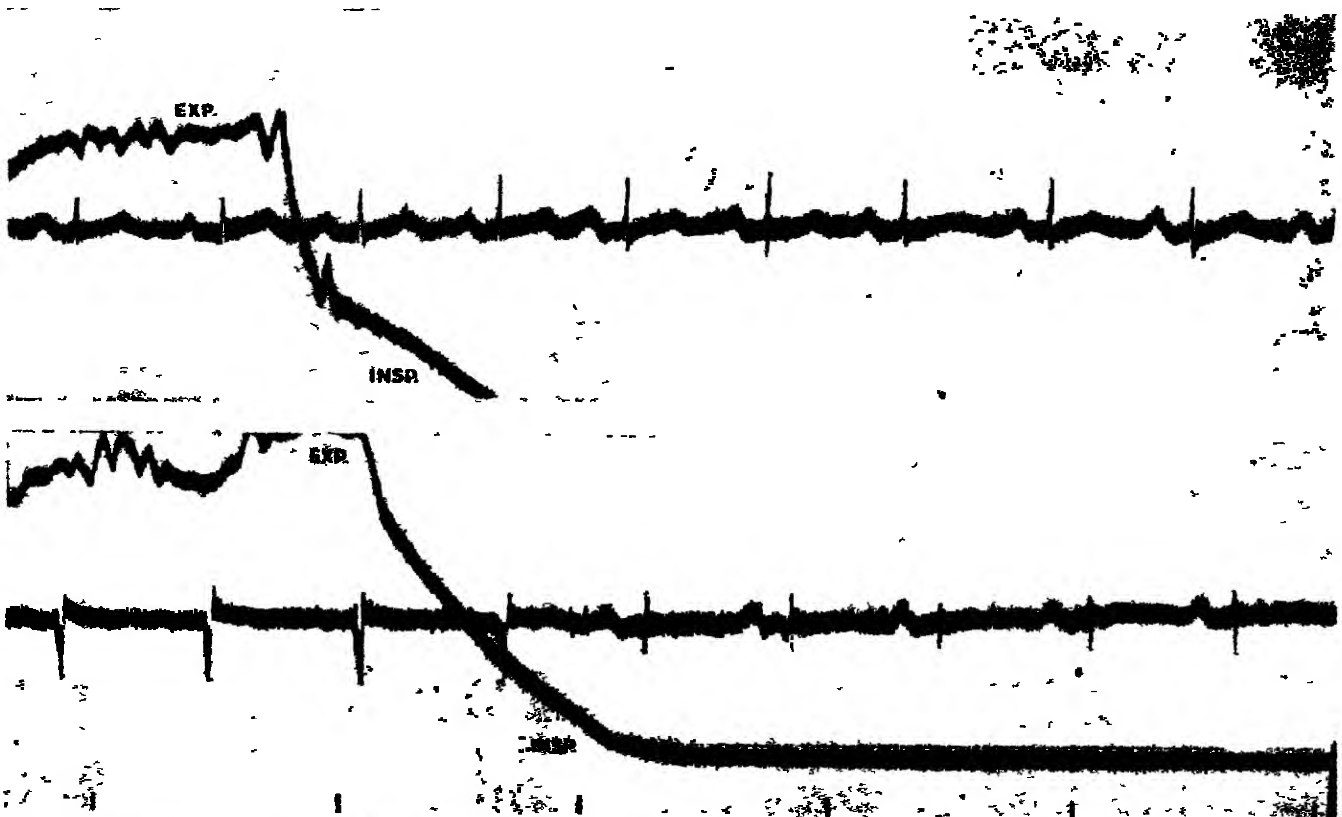

Fig 7 —The Case of A D W Experiment 1 A horizontal heart Effects of deep inspiration upon the right and left lateral (inferior) records

The right lateral spike V_1 is increased during deep respiration

The left lateral spike V_1, which is normally negative in this subject, is rendered positive during deep inspiration At the same time the auricular peak *a*, which is positive, is augmented

The axial angle α, calculated from the R and L values of V_1 comes out as $= 100°$ in extreme expiration and $= 22°$ in extreme inspiration.

FIG. 8.—THE CASE OF J. C. W Experiment 2 An oblique heart. Effect of extreme expiration upon the right and left lateral (inferior) records.

The right lateral spike V_I is slightly increased, then diminished

The left lateral spike V_I, normally positive, is rendered negative by extreme expiration

The inferior axial angle a, calculated from the R and L values of V_I comes out as $= 12°$ in extreme inspiration, and $= 86°$ in extreme expiration.

fluctuation produces in the mind of the observer a corresponding state of indecision as to what value is to be taken for difference of potential during the three to four hundredths of a second of the presystole while the chemical and electrical changes preparatory to contraction are proceeding I have given much thought to such doubtful cases, and made many attempts to synchronise fluctuating records and to calculate the fluctuating angle at definite points during the initial period of systole, but without any satisfactory result, the fluctuations have proved to be too rapid to allow of any satisfactory establishment of corresponding points in time between different records, even when such records have been taken simultaneously I have therefore for the present abandoned the attempt, and taken for these doubtful cases maximal and minimal values, whether positive or negative, and calculated separate values of α from such values, duly noting, of course, their doubtful character. An example in point is given in Part I at p 520, the case of Dr. E.

I have not found it possible to make any satisfactory correction for asynchronism between the initial and culminating points of the transverse and lateral spikes, and have taken into formula only the values of the right and left lateral (inferior) spikes, of which, according to my observations, the asynchronism is so small as to be negligible]

Values of V_I in the several Leads at Different Values of the Axial Angle (Taken by direct readings of the model to the nearest millimetre)

	I. Transverse.	II Right inferior	III Left inferior	IV Right superior	V Left superior	VI Longitudinal
0°	0	100	100	50·0	50	150
10	17	107	90	40 5	58	148
20	34	111	77	30·0	64	141
30	50	111	62	18 0	68	130
40	64	109	45	6 0	70	115
50	77	102	26	− 6 0	70	96
60	87	93	7	−18 0	68	75
70	94	81	−18	−30·0	64	51
80	98	66	−32	−40 5	58	26
90	100	50	−50	−50·0	50	0

Values of V_I in the Inferior Leads, taking into reckoning a Difference of Potential between the two Feet

	Left inferior		Right inferior		Left longitudinal	Right longitudinal	Inferior transverse.
	Left lateral	Equatorial	Right lateral	Axial			
0°	100	100	100	100	150	150	0·0
10	91	89	106	108	149	147	1·7
20	79	75	109	113	143	139	3·4
30	64	58	109	118	132	128	5·0
40	48	41	105	111	118	112	6·4
50	30	22	99	106	100	93	7·7
60	11	2	89	98	79	71	8 7
70	−8	−18	76	86	56	47	9 4
80	−27	−37	62	72	31	21	9 8
90	−45	−55	45	55	5	−5	10 0

I have repeated these two experiments many times and have rarely failed to bring off either the first or the second upon the subjects who have submitted themselves to one or other of the two I have failed to effect complete reversal in only one or two cases where the electrical axis was above the horizontal or very nearly vertical

With a slight modification the model given in fig 5B serves to indicate the nature and amount of the slight differences observable between the two pairs of inferior leads in consequence of the slight differences of potential that are produced at the two feet by the systolic spike V_I The potential-difference between the two feet in what may be termed the inferior transverse lead is in the same direction as that of the hand-to-hand potential-difference, but of much lower value The inferior transverse = 1/20 to 1/5 of the (superior) transverse; as an average for the purpose of calculation it is taken here = 1/10, and represented on the fig 5B by the horizontal line *rl* 1 cm. long across the point F

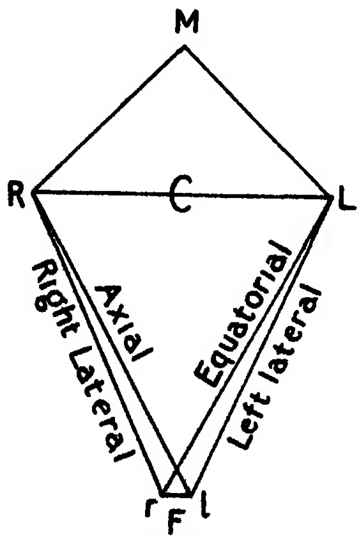

Fig 5B

If now the positions of the two points *r*, *l* (representing right and left foot), are calculated for values of α from 0° to 90°—or pricked off on the model—and plotted as before, the curves given in fig 9 are obtained showing

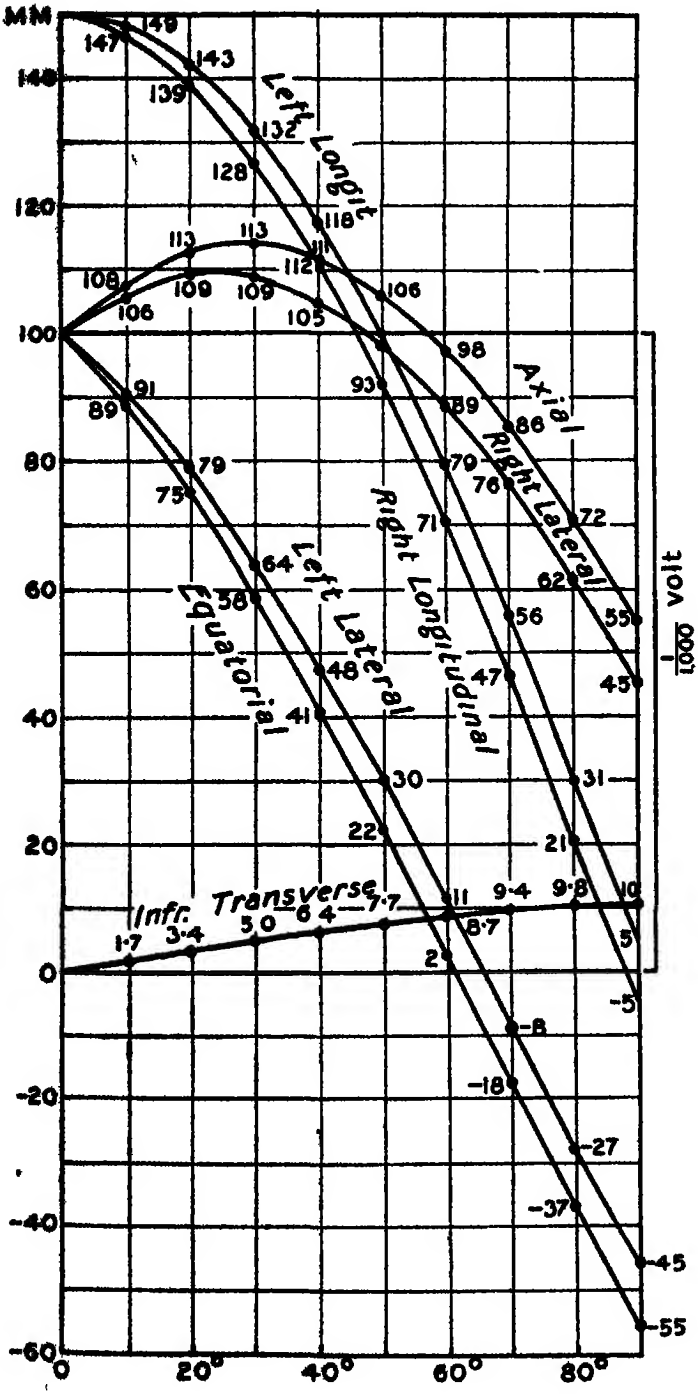

FIG 9—Curves constructed in a similar manner as those given in fig 6, to show the slight differences of the electromotive value of the spike V_1 in the inferior transverse lead and in the two right inferior leads (axial and right lateral) and in the two left inferior leads (equatorial and left lateral) The numbers along the curves in this fig as in fig 6 express 1/100000ths of a volt, *e g*, 108 signifies 0 00108 volt

clearly the theoretical values of the differences in the several inferior leads—axial and right lateral, equatorial and left lateral—which in fig. 5A were assumed to be identical under the designations right inferior and left inferior on the assumption that the two feet might be regarded as isoelectric.* By reference to this figure it is easy to satisfy himself what order of error can arise by regarding the two feet as isoelectric, and to understand without effort certain minor but otherwise puzzling discrepancies met with in certain hearts when the several inferior leads are compared by means of a highly sensitive instrument. Thus *e.g.* it is obvious at a glance that such discrepancies become more sensible in "horizontal" than in "vertical" cases, and that *e.g.* a 64° heart offers the paradoxical instance of a positive left lateral and a negative equatorial spike. And the observation which I have frequently made without thoroughly understanding it to the effect that an equatorial spike is smaller than the left lateral when positive, but the larger of the two when negative, is rendered obviously intelligible. Likewise that mentioned without comment in Part I, p. 510, of this paper that the left is slightly larger than the right longitudinal spike.†

The two experiments described above can be repeated with advantage in view of this second diagram model, upon consideration of which it will be apparent that in repeating Experiment 1 the left lateral is slightly more favourable than the equatorial lead, and that in repeating Experiment 2 the equatorial is slightly more favourable than the left lateral.

The differences of value between axial and right lateral and between equatorial and left lateral are obviously small, and their absolute measurement involves a large relative error. For instance, in the case of J. C. W. the values come out as follows:—

* In my first communication ('Phil. Trans.,' 1889, p. 191) the two feet are given as being isoelectric, although I was well aware of the fact that theoretically there should be a slight P.D. between them.

† In that connection, speaking only of the four leads between the mouth and four extremities, I used the expression right and left superior and inferior, whereas for right and left inferior I should have used the designations right and left longitudinal, in accordance with the terminology of the present paper, where right and left inferior refer to hands and feet. The table given on p. 510 should read accordingly:—

Right superior .	3	(= 0·00023 volt)
Left superior . . .	15·3	(= 0·00118 ,,)
Right longitudinal ...	16·5	(= 0·00126 ,,)
Left longitudinal	17·5	(= 0·00135 ,,)

Transverse	0 0020 volt.	
Right lateral	0 0024	„
Axial	0 0028	„
Left lateral	0 0015	„
Equatorial	0 0012	„
Inferior transverse .	0 0002	„

Pathological Applications

The various applications of the electro-cardiogram to clinical diagnosis of heart lesions fall into two chief divisions Class A, in which the indications are certain, Class B, in which they are uncertain Class A is represented by the arhythmiæ, no mistake is possible *e g* as to alterations of frequency and rhythm, coupled beats, auriculo-ventricular dissociation Class B includes among many others the supposed electrical signs of right and left ventricular hypertrophy and of partial interruptions of auriculo-ventricular conduction It is as regards Class B that I believe it to be most necessary to pay attention to the physical relations of the normal heart

The electrical signs that are accepted by clinical authorities as associated with right ventricular hypertrophy as expressed in clinical language are "small R_I, large R_{III}," or, as I prefer to express it, small transverse, large left lateral spike These are, as has been explained above, physical evidence of an approximately vertical electrical axis The great majority of clinical observers agree in stating that this combination of small R_I and large R_{III} is common in mitral disease, and that it signifies hypertrophy of the right side of the heart I have seen many cases during the last three years that are in agreement with this statement, but, on the other hand, I have during the last 20 years met with still more numerous cases of apparently perfectly normal persons that presented this combination, and have only inferred that they possessed vertical hearts I have become accustomed to expect to find this combination in infants and in any tall healthy young man accustomed to take plenty of open-air exercise Therefore, without presuming to express any opinion as to the clinical value of this sign of right ventricular hypertrophy, I do venture to say that in the first instance our reasoning from the sign should be limited to the conclusion that in its presence the electrical axis of the heart must be vertical or directed to the right, and bear in mind that this indication is presented by the hearts of many normal persons The diagnosis of right ventricular hypertrophy has to be established on independent clinical grounds.

The electrical signs that are presented as being significant of left ventricular hypertrophy are, in clinical language, large R_I, small or reversed

R_{III}, or, as I express it, a large transverse and a small or reversed left lateral spike. Precisely similar considerations apply to this sign in relation to diagnosis as have been just stated as regards the right side of the heart. I have seen cases that agree with the clinical statement (but others that do not), and I have seen far more numerous cases of normal persons with small transverse and negative left inferior spikes, and have inferred therefrom that the electrical axis of the heart was approximately horizontal. I have met with this sign at all ages and in all conditions of health, and have become accustomed to expect to find it in anæmic young women and in aged persons of either sex. I associate it in my mind with a soft or flabby heart muscle, but possess no confirmatory *post-mortem* evidence of that impression.

The electrical signs that are presented as being significant of interruption of the right (or left) branch of the auriculo-ventricular bundle of Kent and His consist essentially in a reversed and prolonged R_{III} resembling a left ventricular extra-systole, but occurring in sequence to an auricular contraction. All the cases hitherto reported which have been confirmed by *post-mortem* examination have been on the right side, and have been characterised electrically *intra vitam* by a negative left lateral deflection, which has been accepted as an indication of ventricular contraction initiated on the left side. I shall not venture to deny the possible accuracy of the chain of argument upon which the diagnosis of the interruption depends, but in estimating probabilities I think it should be clearly realised that a negative left lateral deflection is of frequent normal occurrence.

In Part I of this paper it has been shown that the inferior angle α varies within a very wide range ($-10°$ to $+100°$) with the shape and position of the heart. A presumably "soft" heart, of which the muscle is deficient in tone, is sessile upon the diaphragm, and its electrical axis is approximately horizontal. With "hard" muscle the heart, even during diastole, is more nearly erect upon the diaphragm, and its axis is more nearly vertical. The axial angle is decreased by inspiration, increased by expiration; it is decreased by muscular exercise, increased by repletion of the stomach. I think that it is extremely probable that to this series of statements it may be added that, pathologically, the angle is decreased by engorgement of the right side of the heart (as occurs *e.g.* in mitral disease), so that the electrical axis may be vertical or actually directed to the right, and increased by hypertrophy of the left side especially (as occurs *e.g.* in aortic disease), so that the axis may become horizontal. But since both these conditions, *i.e.* axis to the right and axis horizontal, are compatible with a normal state, I do think that either a remarkably large left lateral spike or a reversed left

lateral spike is to be admitted as affording *per se* proof of the existence of right or left hypertrophy or dilatation

Considering, further, that a negative left lateral spike is of frequent occurrence in the normal as well as in the diseased heart, it cannot be admitted as affording *per se* proof or even evidence of an interruption of the right branch of the auriculo-ventricular bundle The facility with which in certain hearts a positive left lateral spike can be rendered negative is such as to forbid us from admitting a temporary reversal as an indication of temporary interruption of conduction to the right ventricle

[*Note added May* 23, 1914—By courtesy of Dr Part of the National Hospital for Diseases of the Heart, I am able to complete the account of the cases of A. D W and of J C W by the reduced skiagraphic outlines (fig 10) of their hearts in the positions of deep inspiration and expiration The outlines of the heart and diaphragm indicate approximately the anatomical alterations corresponding with the electrical alterations given in figs 7 and 8 There is, however, in these cases no absolute correspondence of axial angles to be made out between the anatomical and the electrical estimates The skiagrams required the breath to be held for several seconds in inspiration and in expiration respectively In general, the correspondence between the anatomical and the electrical axial angle is not very close As a rule the

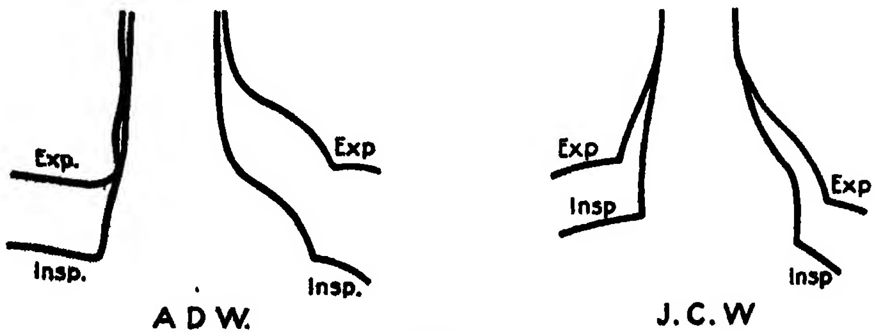

Fig 10

right and left electrical effects of the infantile heart which is mesial may be expected to be about equal, while in the senile heart which tends to become horizontal the left hand inferior spike is more usually negative. But cases occur of apparently normal as well as of diseased hearts (*e.g.* mitral diseases) where the left inferior is larger than the right inferior spike (implying an electrical axis directed to the right and a reversed transverse spike), but where the anatomical axis is distinctly directed to the left. Cases also occur of reversed left inferior spike where the electrical axis comes out

as greater than 90°, *i.e.* directed upwards to the left, but where the anatomical axis is distinctly less than 90°, *i.e.* directed downwards to the left.]

Corrigenda in Part I, 'Roy Soc Proc,' B, vol 86

Page 512 In the second line of the footnote tan = *should read* tan a =

Page 514 Last line, tan $a = \frac{R-L}{R+L}$ *should read* tan $a = 2\frac{R-L}{R+L}$

Page 520 The record of the right superior lead is placed upside down. The first ventricular wave is actually negative

Page 525 The numbers 36 and 47 in the last column (15th and 16th from bottom) should be transposed.

On the Relation between the Thymus and the Generative Organs and the Influence of these Organs upon Growth

By E T Halnan and F. H A Marshall. (With a Note by G Udny Yule)

(Communicated by Prof J. N. Langley, F R S Received April 4,—Read June 18, 1914)

Calzolari was the first to show that in castrated male animals the absolute weight of the thymus is larger than that of the same gland in normal animals The experiments were made upon six rabbits, which were castrated when between one and three months old and killed at various periods afterwards up to nine months, each rabbit being compared with a control Subsequently Henderson carried out a statistical investigation upon the weight of the thymus in cattle, and showed that in these animals castration caused a persistent growth and a retarded atrophy of the gland. Henderson also records two experiments upon guinea-pigs by Noel Paton, and the results of these are confirmatory of the observations upon cattle.

The possible reciprocal action of the thymus upon the testis was investigated by Noel Paton, who removed the former organ from 24 young guinea-pigs and killed them when they attained weights varying from 115 to 355 grm. These animals were compared with 23 normal guinea-pigs kept as controls. The conclusion reached was that in guinea-pigs below 300 grm (*i.e.*, prior to the time when the thymus usually atrophies) thymectomy is followed by a more rapid growth of the testes. In guinea-pigs above 300 grm. Paton found that the difference in weight of the testes in thymusless and normal animals was not manifest. The figures upon

which these conclusions are based are dealt with statistically in an appendix to this paper by Mr G Udny Yule.

Noel Paton's conclusions have been challenged by Soli, who worked upon guinea-pigs and upon fowls. The guinea-pigs, except in the case of two pairs, were killed when weighing considerably over 300 grm, so that the results have no bearing upon Paton's assertion that in guinea-pigs below that weight thymusless animals tend to have larger testes than normal individuals In the two pairs killed below 300 grm the testes of the operated guinea-pigs were slightly lighter than those of the control animals In the experiments upon fowls Soli found that in two cases the thymusless birds had heavier testes than the controls, but in 11 cases the testes of the operated individuals were lighter than those of the unoperated Paton, however, points out that in certain of these the rate of growth was below the normal, and that the small size of the testes might have been due to inferior nutrition. Soli found that castration produced hypertrophy of the thymus or arrested atrophy in that organ In the unoperated birds the average weight of the thymus was 0 6 grm to each kilogramme of body weight, whereas in the capons its weight was 1 16 grm. to each kilogramme of body weight.

Gellin, Klose and Vogt, Marrassini, and Squadrini have also found that castration tends to enlargement of the thymus, or arrests the normal involution of the gland.

As a result of a further series of experiments, Paton has concluded that the thymus and the testis do not act antagonistically to one another, but that each organ has a stimulating influence upon growth, the one organ compensating for the removal of the other by undergoing hypertrophy. Paton found that castration alone without thymectomy had no effect upon the growth of young guinea-pigs, neither had thymectomy alone any influence upon the rate of growth On the other hand, castration and thymectomy performed simultaneously in very young guinea-pigs was found to check growth. Considered in the light of our experiments to be described below we are of opinion that this effect may have been consequent upon the double operation, which very possibly lowered the resistance of the animals towards disease Paton describes further experiments showing that in six castrated males and four castrated females the average weight of the thymus was greater than in control animals.

Basch, and also Klose and Vogt, describe extirpation of the thymus in dogs as producing a softening of the bones or retarding the growth of the bony tissues, besides causing other pathological phenomena. Similar changes resulting from thymectomy are described by Matti Soli also has confirmed

these results for rabbits, but failed to confirm them for guinea-pigs, in which thymectomy is a simple operation. It is not improbable, therefore, that the inhibitory effects of the growth observed by Basch and others were merely post-operative, since they only occurred when the operation of thymectomy was a severe one

Gudernatsch states that tadpoles fed upon thymus extract grew to an abnormal size and postponed undergoing metamorphosis. In some cases they did not change into frogs at all, but remained as giant tadpoles.

Stotsenburg, working upon the effect of ovariotomy on the growth of albino rats, found that this operation caused an increased rate of growth This appeared to be the case not only after the age of sexual maturity was reached, but prior to the attainment of this age, since the removal of the ovaries appeared to induce an accelerating effect forthwith

Lastly, Miss Hewer in a recent paper states that it is possible to induce a hyperthymic condition in rats by feeding these animals upon fresh thymus or upon thymus tabloids, and that this condition is accompanied by partial or complete sterility, the spermatogenetic tissue in the testes ceasing to be active or even undergoing atrophy It is to be noted that these results are directly contrary to Paton's theory of a compensatory mechanism between the thymus and the testis

Record of Experiments with Guinea-pigs.

The experiments described below were undertaken to put on a more quantitative basis the results obtained by Noel Paton in a previous paper, and form, with a few minor differences, a repetition of his work on the subject

In the control thymectomies (pseudo-thymectomies) the thymus glands were exposed without being removed, and the neck afterwards sewn up in the usual way so as to make the operation resemble as nearly as possible an actual thymectomy In the vasectomy experiments a portion of each vas deferens was removed, but the vascular supply of the testes was not interfered with

The guinea-pigs were housed in roomy wire cages and given a liberal diet of oats, bran, and roots, varied occasionally with green food as circumstances permitted The normal animals were housed in the same cages as the experimental ones, thus ensuring uniformity of external conditions for both sets of animals With the exception of one experiment all the animals used were males.

Experiment 1. *Effect of the Removal of the Testes upon the Weight of the Thymus and the Growth of the Animal.*—In this experiment, 12 male guinea-

pigs were used The experiment started on December 20, 1912. On February 7 the testes from six guinea-pigs were removed On March 4 the thymuses of the entire set were extirpated. The animals were killed on May 9 The number of animals taken was too few to establish any possible growth effects due to removal of the thymuses and testes. The animals retaining the testes grew less in the later stages of the experiment, this possibly being due to the castration effect on growth observable in all castrated adult animals. With regard to the effect on the thymus, small though the number of animals is, the evidence is quite clear Moreover, the fact that the animals castrated had all attained puberty before castration took place shows that the arrested atrophy and subsequent hypertrophy of the thymus cannot be explained by Paton's theory

Animal No	Days after first weighing				Weight of thymus
	0	40	74	140	
		Control Animals			
	grm	grm	grm	grm	grm
1	237	355	475	580	0 276
2	192	335	354	—	0 342
3	215	358	442	497	0 370
4	214	342	452	617	0 337
5	202	357	377	588	0 400
Average— 4 animals	—	—	436	570	
5 "	212	349	420		0·345
		Operated Animals			
1	212	355	402	615	0 430
2	240	400	470	682	0 395
3	184	345	375	567	0 470
4	187	360	380	610	0 564
5	225	375	447	674	0 680
Average	209	367	415	629	0 508

Experiment 2 (figs 1, 2, and 3) · *Effect of Removal of the Testes on the Weight of the Thymus, of Removal of the Thymus on the Weight of the Testes, and upon the Growth of the Animal.*—This set contained 10 normal animals, 7 castrated, and 6 thymectomised. The animals were operated upon on February 24–27, and first collectively weighed on March 4. On May 9 the experiment terminated

Reference to the data below shows that thymectomy has no effect upon growth, and that castration has little, if any, effect

Animal No	Days after first weighing		Weight when killed	Weight of testes + epididymes	Weight of thymus
	0	63			
	Control Animals				
	grm	grm	grm	grm	grm
1	202	349	375	1 25	0 253
2	157	365	380	1 43	0 395
3	164	385	420	2 ·05	0 302
4	182	310	320	1 02	0 197
5	127	275	290	0 376	0 390
6	170	412	420	1 67	0 350
7	180	395	410	2 08	0 380
8	185	410	425	1 82	0 300
9	221	452	470	2 38	0 395
10	191	452	468	2 43	0 390
Average	168	380	398	1 651	0 344
	Castrated Animals				
1	214	401	430	—	0 580
2	211	405	420	—	0 750
3	155	320	330	—	0 405
4	185	377	428	—	0 507
5	235	462	485		0 680
6	220	435	470	—	0 ·710
7	204	292	335		0 416
Average	203	385	413	—	0 578
	Thymectomised Animals				
1	135	440	450	2 185	
2	154	395	410	1 560	
3	154	315	325	1 200	
4	167	384	390	1 705	
5	142	347	355	0 ·835	
6	183	394	400	1 260	
Average	156	379	388	1 457	

Experiment 3 (fig 1) *Effect of the Removal of the Testes on the Weight of the Thymus and the Growth of the Animal*—This set contained originally 7 castrated and 9 normal animals, but owing to deaths only 4 castrated and 6 normals are recorded The experiment commenced on the day of operation, March 17, and finished on June 24 Here castration seems to have had a positive effect upon growth, but the number of castrated animals (4) indicates the tentative nature of this result As in Experiments 1 and 2 the effect of castration on the weight of the thymus is well marked

Animal No	Days after first weighing — 0	96	Weight of testes + epididymes	Weight of thymus
		Control Animals		
	grm	grm	grm	grm
1	93	283	1 225	0 190
2	109	322	1 235	0 265
3	107	285	0 870	0·175
4	165	420	2 100	0 295
5	109	386	2 154	0 217
6	165	390	1 750	0 275
Average	124	339	1 556	0 246
		Castrated Animals		
1	148	412	—	0 610
2	140	346	—	0 452
3	111	372	—	0·430
4	124	391	—	0 540
Average ..	131	380	—	0 508

Experiment 4 (fig 1). *Effect of the Removal of the Testes on the Weight of the Thymus and on the Growth of the Animal*—To annul the operative effect the control animals were vasectomised. The experiment started on May 28 and finished on August 1 with 6 vasectomised and 5 castrated animals. The evidence in this experiment with regard to growth is contradictory to Experiment 3

Animal No	Days after first weighing. 0	65	Weight of thymus.
	Vasectomised Animals		
	grm	grm	grm
1	245	387	0 488
2	230	424	0 400
3	268	467	0 500
4	173	282	0 264
5	203	373	0 300
6	170	400	0 398
Average	215	389	0 380
	Castrated Animals		
1	243	330	0 578
2	280	405	0 607
3	210	353	0 738
4	147	320	0 545
5	157	336	0 614
Average	207	349	0 615

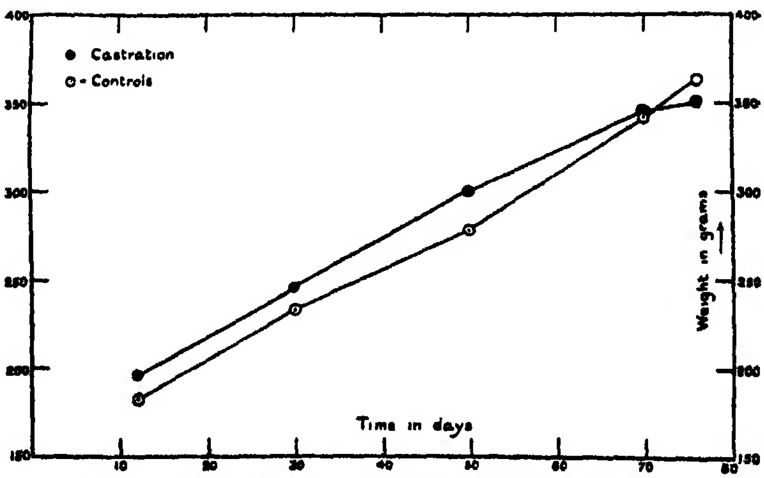

Fig 1.—Effect of Castration on the Growth of Guinea-pigs.

(The curves are formed from the averages of the individuals of Experiments 2, 3, and 4.)

Experiment 5 (fig 2) *Effect of the Removal of the Thymus on the Weight of the Testes and on the Growth of the Animal*—To annul the operative effect the control animals were pseudo-thymectomised. The experiment started on May 27 and finished on July 8 There were 7 thymectomised animals and 6 pseudo-thymectomised in this set The results obtained support the contention that thymectomy has no effect upon growth.

Animal No	Weight, May 27.	Weight after 42 days	Weight of testes + epididymes	Weight of thymus
		Control Animals		
	grm	grm	grm	grm
1	210	325	1 420	0 325
2	177	316	1 155	0 510
3	190	267	0 610	0 315
4	151	238	0 470	0 235
5	162	290	1 012	0 320
6	190	338	1 380	0 283
Average	180	296	1 008	0 331
		Thymectomised Animals		
1	141	265	0 770	
2	227	307	1 112	
3	128	288	0 313	
4	158	257	0 574	
5	197	287	0 880	
6	150	245	0 527	
7	187	330	1 363	
Average	169	276	0 791	

Experiment 6 (fig 2) *Effect of the Removal of Thymus on the Weight of the Testes and on the Growth of the Animal*—Nine normal animals and 14 thymectomised were used in this set The operations were performed June 19–26. The experiment started on July 1 and ended on July 25 The evidence supports Experiment 5 with regard to growth The effect on the testes is here in accordance with Noel Paton's results.

Animal No.	Weight, July 1.	Weight after 24 days	Weight when killed	Weight of testes	Weight of thymus
			Control Animals		
	grm	grm	grm	grm	grm.
1	133	187	200	0 320	0 277
2	213	317	322	1 250	0 410
3	201	267	272	1 120	0 322
4	151	245	252	0 676	0 350
5	136	193	207	0 320	0·320
6	171	245	235	0 620	0 408
7	226	316	340	1 125	0 480
8	170	268	307	0·970	0 508
9	166	252	240	0 648	0 460
Average	174	254	264	0 781	0 392
			Thymectomised Animals		
1	283	347	352	1 43	
2	156	219	221	0 454	
3	238	358	380	1 800	
4	168	226	212	0 425	
5	167	215	235	0 580	
6	201	260	245	0·930	
7	168	233	215	0 620	
8	205	296	300	1·170	
9	157	238	258	0·623	
10	197	301	310	1 450	
11	225	316	364	1·702	
12	205	256	237	0 835	
13	152	230	265	0 640	
14	158	234	257	0 630	
Average	187	266	275	0·949	

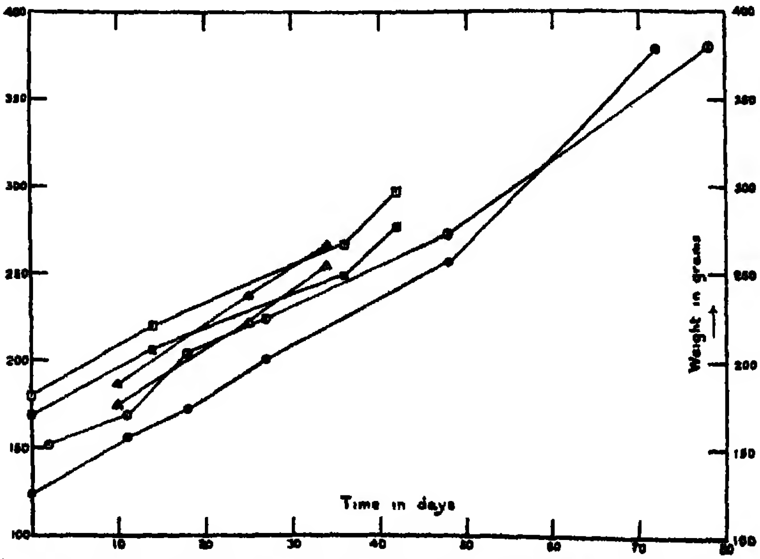

FIG 2.—Effect of the Removal of the Thymus on the Growth of Guinea-pigs.

(The squares represent the averages of Experiment 5, the triangles Experiment 6, and the circles Experiment 2 In all cases the blacked figures represent the operated animals.)

Experiment 7 (fig 3): *Effect of the Simultaneous Removal of the Testes and Thymus on the Growth of the Animal*—This experiment was undertaken to investigate the effect of castration and thymectomy together on growth In order to annul the operative effect the controls were vasectomised and pseudo-thymectomised. The experiment commenced with 9 animals, 4 operated, 5 controls, on July 16, and with 16 others (8 a side) on July 25. Reference to the protocol will show that this double operation has no effect upon the growth of guinea-pigs, a result directly contrary to that of Noel Paton

Set 1

Animal No	Weight, July 16	Weight after— 9 days	37 days	75 days	86 days	101 days	126 days	160 days	177 days
Control Animals									
	grm	grm	grm	grm	grm	grm	grm	grm	grm
1	180	210	306	342	338	357	437	542	607
2	178	202	270	290	265	292	328	433	460
3	185	216	300	372	370	402	442	543	582
4	192	220	301	340	340	347	397	489	509
5	105	139	287	286	285	317	388	455	485
Average	168	197	283	326	319	343	397	492	528
Operated Animals									
1	186	215	280	335	344	360	375	445	472
2	195	221	285	331	320	345	387	474	502
3	160	191	282	318	319	322	367	445	492
4	197	228	311	343	354	345	370	480	465
Average	184	212	289	332	334	343	375	461	483

Set 2

Animal No	Weight, July 25	Weight after— 28 days	66 days	79 days	92 days	116 days	141 days	168 days
				Control Animals.				
	grm	grm	grm	grm	grm	grm	grm	grm
1	155	278	352	337	365	408	512	537
2	160	250	343	359	367	448	525	555
3	260	231	248	272	290	334	407	445
4	183	212	286	285	300	378	450	417
5	155	259	355	368	370	450	534	574
6	120	221	276	287	312	353	377	357
7	220	321	395	393	394	452	522	578
8	156	250	336	330	340	419	478	475
Average	174	253	330	329	342	405	475	490
				Operated Animals				
1	181	257	318	323	327	382	428	490
2	190	286	346	368	382	487	480	552
3	136	235	291	302	327	358	407	390
4	175	208	340	337	340	400	450	487
5	156	270	377	388	410	430	517	570
6	173	250	268	273	297	327	383	395
7	170	277	386	380	410	485	512	567
8	212	275	336	346	287	379	423	470
Average	174	264	333	338	347	393	450	490

Experiment 8 (fig 3) *Effect of the Simultaneous Removal of the Testes and Thymus on the Growth of the Animal*—The animals in this experiment were treated as in the previous experiment, the operations extending from July 30 to August 5 The first collective weighing took place on August 22 There were 5 operated and 5 control animals The results obtained in this experiment confirm the findings in Experiment 7

Animal No	Weight, Aug 22	Weight after— 38 days	51 days	64 days	88 days	113 days	140 days	152 days
				Control Animals				
	grm	grm.	grm	grm	grm	grm	grm	grm
1	208	309	340	397	495	498	577	590
2	285	374	396	422	484	572	537	547
3	278	320	335	355	452	520	567	548
4	157	146	180	232	319	409	465	465
5	183	330	360	380	462	587	585	575
Average	221	296	322	357	442	507	546	545
				Operated Animals				
1	243	382	435	407	469	537	607	622
2	135	176	177	235	284	354	350	360
3	230	373	405	435	487	552	607	600
4	152	286	320	365	402	485	520	565
5	217	360	402	387	482	525	606	615
Average	195	315	347	366	425	491	538	552

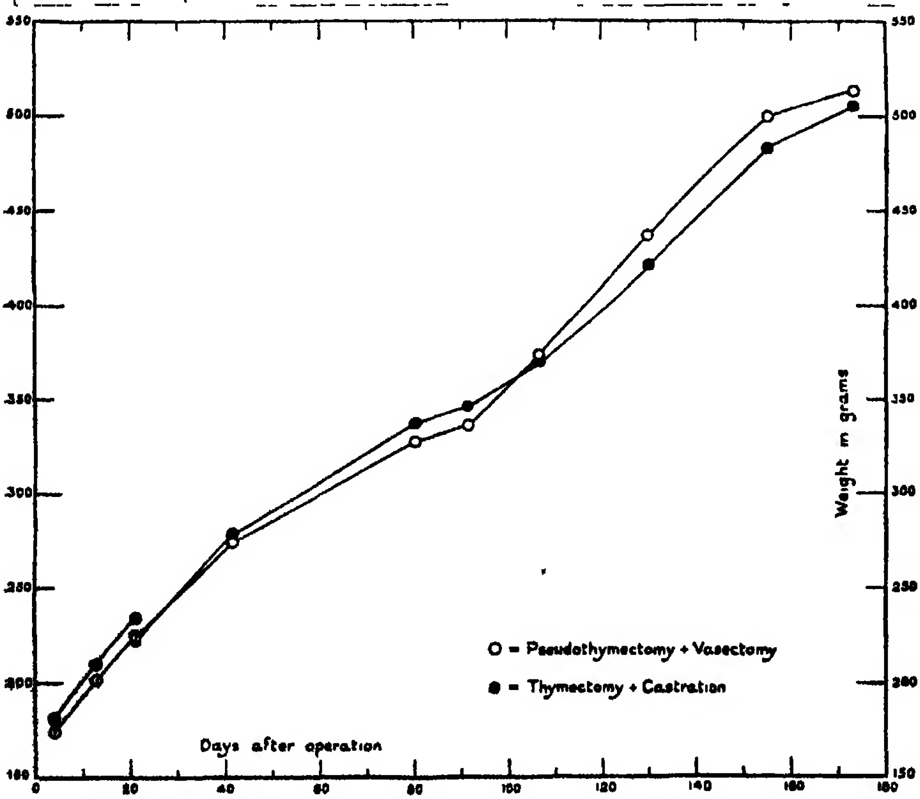

Fig. 3.—Effect of Thymectomy and Castration on the Growth of Guinea-pigs

(The curves are produced from Experiments 7 and 8, the averages of Experiment 8 and of the second set in Experiment 7 being interpolated to give the approximate average weights on the desired intervals after operation The separate portion at the beginning of the curve is due to the fact that the weighings of certain animals only commenced 20 days after operation)

Experiment 9 (fig 4)· *Effect of Semi-Castration on the Weight of the Thymus.*—In this set 7 animals were semi-castrated The results, in so far as these related to the thymus weights, were too variable to admit of any importance being attached to them

Animal No	Weight, August 13	Weight after 51 days	Weight of remaining testis	Weight of thymus.
	grm	grm	grm	grm
1	117	310	0 652	0 355
2	184	358	1 214	0 480
3	136	350	1 ·048	0 380
4	117	363	1 206	0 550
5	154	362	1 312	0 385
6	123	368	1 100	0 546
7	148	356	1 400	0 252
Average	133	352	—	0·421

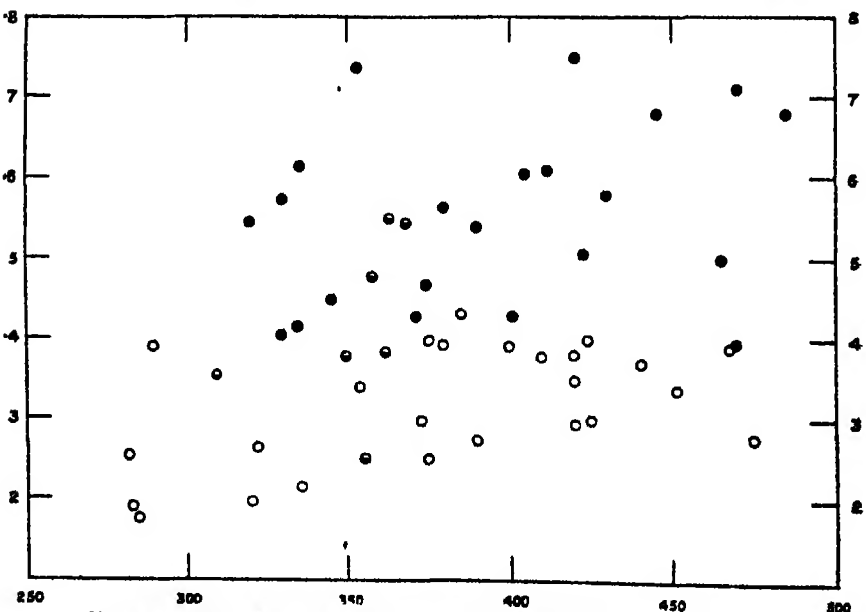

Fig 4.—Effect of Removal of Testis on the Weight of the Thymus

(Blacked circles, castrated animals, semi-blacked circles, semi-castrated animals; clear circles, control animals. Vertical, weight of thymus in grammes, horizontal, body weight in grammes.)

Experiment 10: *Effects of the Removal of the Ovaries on the Weight of the Thymus*—Five females were castrated August 11–14, and compared with 6 controls The experiment extended from August 11 to October 9 Reference to the data given will show that castration in the female, as in the male, leads to an arrested atrophy and continued growth of the thymus gland

Animal No	Weight, August 11	Weight after 59 days	Weight of thymus
	Control Animals		
	grm	grm	grm
1	182	384	0 305
2	170	246	0 190
3	160	326	0 363
4	177	320	0 280
5	158	365	0 400
6	181	350	0 320
Average	171	323	0 309
	Operated Animals		
1	162	424	0 805
2	160	326	0 405
3	158	406	0 720
4	170	415	0 780
5	145	341	0 590
Average	158	382	0 660

Experiment 11 (fig 5) *Effect of Removal of the Thymus on the Weight of the Testes and on the Growth of the Animal*—Nine animals thymectomised October 21, 9 controls The experiment extended from October 21 to November 18, by which time 6 operated animals and 7 normal animals were over 300 grm in weight The evidence here confirms the findings of Experiments 2, 5 and 6

Animal No	Weight, Oct 21	After 28 days	Test and epid	Thymus.
		Control Animals		
	grm	grm	grm	grm
1	277	332	1·650	0 380
2	253	308	0·820	0 380
3	217	233	0·340	0 120
4	298	335	1 065	0 290
5	205	282	0 520	0 375
6	260	315	0 770	0 340
7	348	392	2 556	0 290
8	272	370	1 695	0 250
9	312	350	2 230	0 235
Average	271	324	1 294	0 284
		Operated Animals		
	grm	grm	grm	
1	270	342	1·010	
2	277	350	1 174	
3	227	303	1 535	
4	287	281	0 544	
5	207	278	0 470	
6	320	355	1 186	
7	370	425	2 504	
8	276	362	1 610	
9	240	298	1 125	
Average	275	332	1 239	

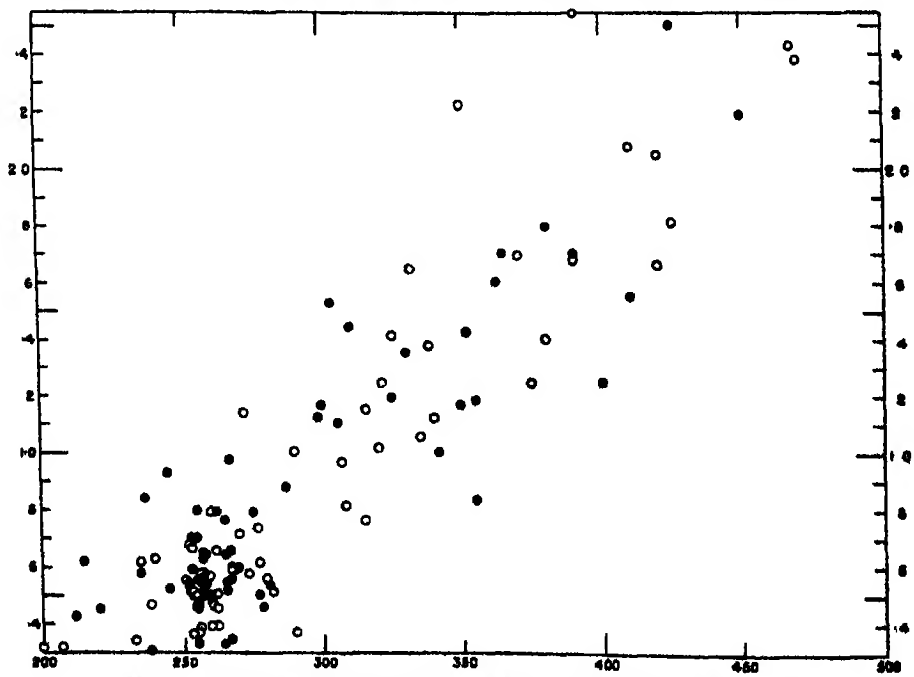

Fig. 5 —Effect of Removal of Thymus on the Weight of the Testis.
(Clear circles, control animals ; black circles, operated animals. Vertical, weight of testes and epididymes in grammes ; horizontal, weight of animal in grammes.)

Experiments 12 to 17 *Effect of the Removal of the Thymus on the Weight of the Testes*—In these experiments, the chief intention was to investigate the effect of thymectomy on the weight of the testes In order to cut out the enormous variation in the weight of the testes obtained as the animal approaches puberty all animals were killed when they attained the weight of about 260 grm

Reference to fig. 5, on which the results obtained from this and other experiments are plotted, shows that thymectomy does not lead to a compensating acceleration in the growth of the testes

Experiment	Animals used		Initial average weight in grammes	
	Normals	Operated	Normals	Operated
12	8	6	188	166
13	—	5	—	164
14	7	5	185	191
15	6	5	139	145
16	9	7	166	187
17	—	4	—	174

Experiment 12

	Initial weight	Final weight	Weight of testes	Initial weight	Final weight	Weight of testes
	Control Animals			Operated Animals		
	grm	grm	grm	grm	grm	grm
	187	277	0 620	153	257	0·550
	222	270	0 720	207	268	0 590
	170	254	0 465	174	275	0 795
	175	254	0 514	138	266	0 550
	187	255	0 456	162	255	0 460
	202	267	0·603	162	257	0 655
	200	267	0 590			
	163	260	0 795			
Average	188	263	0 598	166	263	0 600

Experiment 13.

	Initial weight	Final weight	Weight of testes
Operated Animals			
	grm	grm	grm
	165	260	0·480
	170	277	0·516
	135	266	0·490
	160	269	0·600
	190	257	0·580
Average	164	266	0·533

Experiment 14

	Initial weight	Final weight	Weight of testes	Initial weight	Final weight	Weight of testes
	Control Animals			**Operated Animals**		
	grm	grm	grm	grm	grm	grm
	137	261	0·470	210	258	0·540
	177	255	0·370	227	267	0·568
	240	252	0·520	182	258	0·512
	177	256	0·385	172	255	0·710
	197	253	0·370	165	267	0·663
	—	252	0·545			
	—	258	0·572			
Average	—	255	0·462	191	261	0·598

Experiment 15

	Initial weight	Final weight	Weight of testes	Initial weight	Final weight	Weight of testes
	Control Animals			**Operated Animals.**		
	grm	grm	grm	grm	grm	grm
	165	262	0·460	155	253	0·710
	108	262	0·660	107	253	0·590
	107	253	0·670	210	255	0·560
	130	277	0·505	132	257	0·520
	165	251	0·564	120	252	0·580
	160	262	0·390			
Average	139	261	0·541	145	254	0·582

Experiment 16

	Initial weight	Final weight	Weight of testes	Initial weight.	Final weight	Weight of testes
	Control Animals			Operated Animals		
	grm	grm	grm	grm	grm	grm
	128	258	0 390	132	267	0 350
	150	253	0 520	209	267	0 976
	129	254	0 510	207	264	0 330
	128	255	0 710	215	255	0 330
	160	262	0 520	167	255	0 800
	135	256	0 580	202	259	0 507
	219	273	0 580	183	255	0 480
	236	278	0 740			
	215	279	0 570			
Average	166	263	0 569	187	260	0·539

Experiment 17

Initial weight	Final weight	Weight of testes
Operated Animals		
grm	grm	grm
198	253	0 503
172	262	0 790
152	255	0 465
—	253	0 680
174	256	0 600

Summary of Conclusions

From the evidence given in the above set of experiments, where, in investigating growth effects, the authors were careful to compensate for any possible operative effects, are drawn the following conclusions —

(1) Removal of the thymus in young guinea-pigs does not affect the growth of the animals

(2) Removal of the testes and epididymes in young guinea-pigs does not affect the growth of the animals before sexual maturity

(3) Simultaneous removal of the testes and thymus in young guinea-pigs does not affect the growth of the animals before sexual maturity

(4) Thymectomy is not followed by hypertrophy of the testes.

(5) Castration leads to an arrested atrophy and subsequent hypertrophy of the thymus gland, as found by other investigators.

(6) There is no evidence of the existence of a compensatory mechanism between the testes and the thymus.

The work was carried on at the Field Laboratories, Cambridge. The operations were done by F H A Marshall, the weighings and the chief part of the other work by E T Halnan The expenses were defrayed by a grant made by the Board of Agriculture and Fisheries out of funds placed at their disposal by the Development Commission

Note by G Udny Yule

In view of the disagreement with Prof Paton's conclusions, Dr Marshall asked me to investigate the probable errors of some of the comparisons made, with especial reference to the alleged effect of extirpation of the thymus on the growth of the testes

The problem was not an easy one A glance at Prof Paton's figures, or at the corresponding data given by Halnan and Marshall, will show how exceedingly variable are the weights of the testes and how much caution must consequently be used before basing any conclusion on a small difference between the average weights for two groups of some 20 to 30 animals. Considerable differences might be shown even by the averages of groups treated in precisely the same way Were the animals adult, the "probable error" of the difference between any two observed averages—the amount which it would be as likely as not to exceed owing to mere fluctuations of sampling—might be readily obtained in the ordinary way But the animals are not adult, the weight of the testes increases very rapidly with the weight of the animal, and the weights of the different individuals themselves vary greatly, so that the two groups of operated and controls are not strictly comparable as a whole

What I finally decided to do, therefore, was this · To obtain, by known methods, equations expressing as closely as possible the relation between mean weight of the testes and body-weight, for operated and for normal animals, and to see whether the constants in these equations differed more than could be expected owing to the chances of sampling alone. As in Prof Paton's data the weight of testes did not seem to be a linear function of the body-weight, and it was these data that I first investigated, the logarithm of the testes-weight was substituted for the actual value, and this seemed to give an approximately linear relation, judging from the diagram (fig 6) The two equations, with the probable errors of the constants which I finally obtained from Prof Paton's data, including all the 23 normal

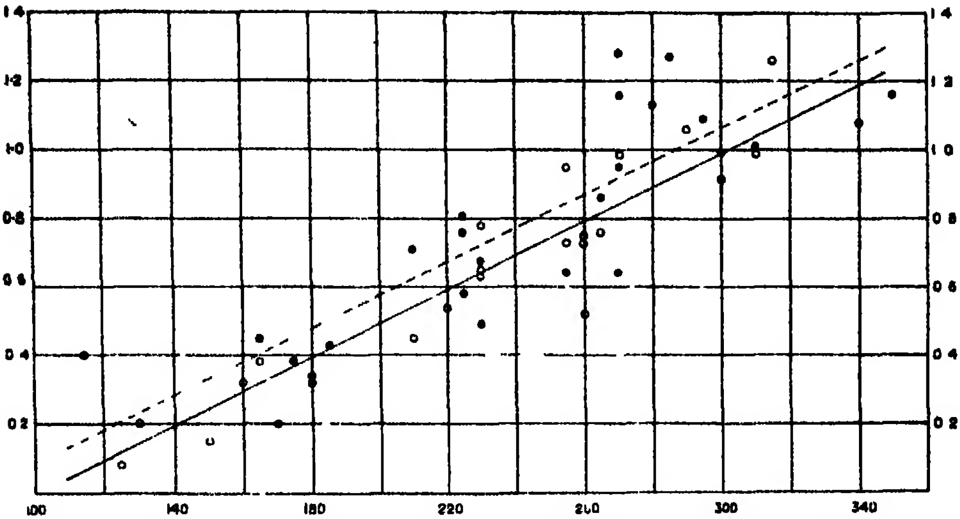

FIG 6 —Effect of Removal of the Thymus on the Weight of the Testes (Prof Paton's data).

(Vertical, logarithm of the testes-weight in decigrammes, horizontal, body-weight in grammes Unbroken line, regression line for normal animals, broken line, regression line for operated animals Clear circles, normal animals, black circles, operated animals)

and 24 thymusless animals, were, t being the testes-weight in decigrammes and b the body-weight in grammes —

Thymusless ..	$\log t = (0\,00489 \pm 0\,00043)\,b - 0\,4069 \pm 0{\cdot}1065$,
Controls	$\log t = (0\,00503 \pm 0\,00025)\,b - 0\,5193 \pm 0\,0608$

If the three normals and four thymusless whose body-weights are over 300 grm be excluded, the results are —

Thymusless	$\log t = (0\,00539 \pm 0\,00051)\,b - 0\,5097 \pm 0\,1203$,
Controls .	$\log t = (0\,00501 \pm 0\,00030)\,b - 0\,5156 \pm 0\,0815$

The probable errors must not be regarded as too precise, since they are obtained on the assumption of normal correlation, but they are likely to give a fair guide to the possible magnitude of fluctuations That of the coefficient of the body-weight (the regression of the logarithm of the testes-weight on body-weight) is the known value

$$0\,6745 \,.\, \frac{\sigma_1\sqrt{(1-r^2)}}{\sigma_2\sqrt{n}},$$

while for the constant term I find the probable error

$$0{\cdot}6745\left(\frac{\sigma_1^2}{n}(1-r^2)+\bar{x}_2^2\,\frac{\sigma_1^2(1-r^2)}{\sigma_2^2 n}\right)^{\frac{1}{2}}$$

where σ_1 and σ_2 are the standard deviations of $\log t$ and body-weights respectively, r is the correlation between them, n is the number of observations, and $\bar{x}_2$ is the mean body-weight Further, it may be noted that there is a high negative correlation between errors in the regression and in the constant term It is clear from the probable errors given that no stress can be laid on the differences observed, which lie well within the range of differences likely to occur owing to fluctuations of sampling alone, equally unlikely or more unlikely differences might have occurred, I find, even had *both* groups been normal, once in some seven or eight trials.

Applying the same method to Halnan and Marshall's data, I find for all the 65 controls and 70 thymusless animals —

Thymusless	$\log t = (0\,00319 \pm 0\,00020)\,b - 0\,0384 \pm 0\,0597$,
Controls .	$\log t = (0\,00367 \pm 0\,00015)\,b - 0\,2032 \pm 0\,0441$,

and for the 43 controls and 49 thymusless under 300 grm,

Thymusless	$\log t = (0\,00210 \pm 0\,00069)\,b + 0\,2195 \pm 0\,1775$,
Controls .	$\log t = (0\,00364 \pm 0{\cdot}00057)\,b - 0\,2098 \pm 0\,1465$.

The difference between the constant terms in this last case looks large, but the probable errors are also very large, and the difference is less than twice its probable error, viz, 0 2301 Summarising in the same way as before, I find differences as improbable as those observed might have arisen owing to fluctuations of sampling once in some five or six trials Halnan and Marshall's data, it may be noted, do not include any animals under 200 grm. and few under 250 grm. and give a low correlation between body-weight and log (testes-weight) for the rather narrow available range of the non-adults. Within the short range of body-weight 250-259 grm, there are 22 thymusless and 17 controls, and it may be desirable to give a simple comparison for these to emphasise the magnitude of the probable errors For controls the mean testes-weight is 0 569, with standard deviation 0·101 grm, for thymusless, mean 0 519, with standard deviation 0·103 The difference, 0 050, is therefore in the direction indicated by Prof Paton's views, but no stress can be laid on it, as it is only 2 25 times the probable error of the difference, viz, 0·0222.

Taking Paton's and Halnan and Marshall's data as a whole then, it seems impossible to regard any effect of extirpation of the thymus on the growth of the testes as proved; if there is any such effect it seems clear that it is small. The data stand in complete contrast with those relating to the effect of castration on the growth of the thymus Within the limits of body-weight in Halnan and Marshall's data, there seems to be little relation between weight of thymus and body-weight, so the means may be compared directly. I find :—

21 castrated animals—mean thymus weight, 0·557 grm., s.d., 0·1104 grm.
27 controls—mean weight of thymus, 0·331 grm.; s.d., 0·0785 grm.

The difference is 0·226 grm., and is 11·8 times the probable error of the difference, viz., 0·0192.

In the case of Prof. Paton's data respecting the effect of simultaneous removal of the thymus and the testes on the rate of growth, the differences observed between operated and control animals seem to point to definite causation. If his Tables III and IV are pooled together, giving 9 operated animals and 12 controls, the difference between the mean gains in weight (viz., 92·2 and 149·2 grm.) is 4·1 times its probable error. For Lot 4 of Table V there are only five animals a side, but the results are unusually uniform. The mean gains in weight are 65 and 120 grm., and the difference 6·6 times its probable error. This result seems in direct conflict with Halnan and Marshall's experiments: they have pointed out above a possible cause for the divergence.

In the preceding, one or two results in probable errors have been given without proof. I hope to publish the proof elsewhere shortly.

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The Cultivation of Human Tumour Tissue in Vitro.—*Preliminary Note.*

By David Thomson, M.B., Ch.B. (Edin.), D.P.H. (Cantab.), Grocers' Research Scholar, and John Gordon Thomson, M.A., M.B., Ch.B. (Edin.), Beit Memorial Research Fellow.

(Communicated by Sir Ronald Ross, K.C.B., F.R.S. Received April 4,—Read May 14, 1914.)

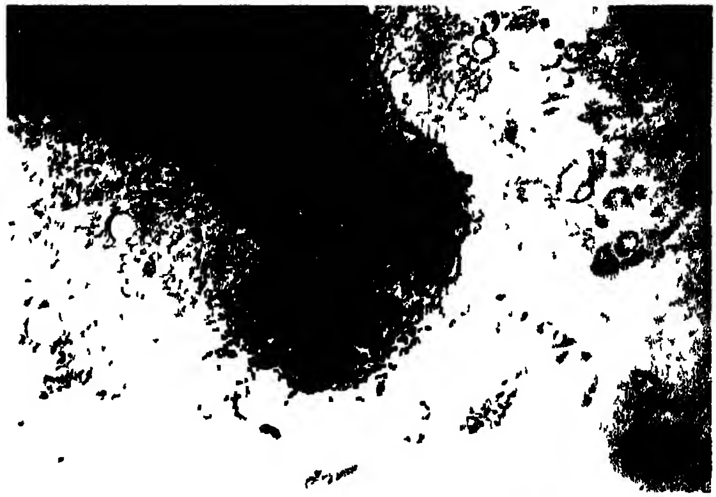
(From the Marcus Beck Laboratory, Royal Society of Medicine, London.)

[Plate 7.]

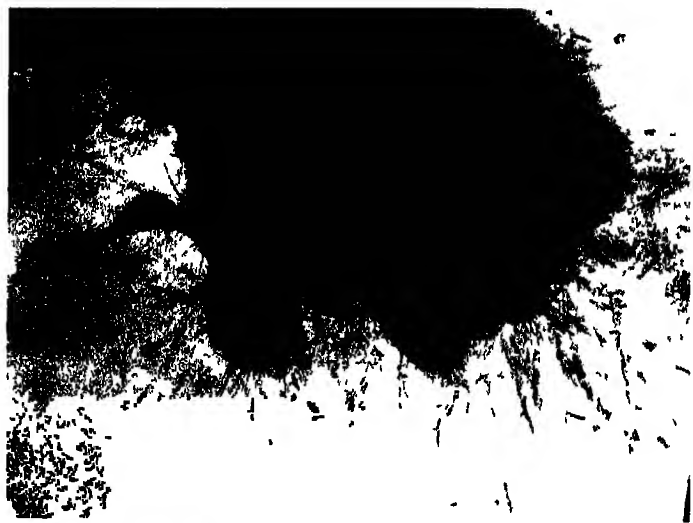
On two occasions the authors have definitely succeeded in cultivating human tumour tissue *in vitro*. The tissue was obtained at operations performed by Sir John Bland-Sutton at the Middlesex Hospital, and conveyed in sterile Ringer's solution in a thermos flask to the laboratory, where small portions were immediately inoculated into the culture medium.

(a) *Intracystic Papilloma of the Ovary* (not truly malignant).—This tissue was grown in a medium composed of fowl plasma 1 part, Ringer's solution (containing 0·5 per cent. of glucose) 1 part, and extract of the tumour in Ringer's solution 1 part. On the third day of incubation at 37·5° C. definite buds of new growing tissue appeared. On the fifth day these were more distinct and on the eighth day the amount of growth had increased considerably (fig. 1, Plate 7). This growth consisted of a solid extension of epithelial cells. As the growth increased it caused some liquefaction of the medium, which was of a gelatinous consistence, and in the more liquefied parts the new growing cells were scattered (fig. 2), but as a rule they remained in contact with each other by means of long fine protoplasmic connections (fig. 3). The new actively proliferating cells varied markedly from the cells of the original tissue planted in the medium. The former were large and amœboid, with long processes which communicated with each other, and they also contained large highly refractile granules. The original cells, on the other hand, were much smaller; they showed no amœboid processes, did not exhibit amœboid movement and they contained few or no refractile granules. This tumour was a very soft one and appeared to contain little or no fibrous stroma. It was composed entirely of epithelial cells, and it will be noted that the new growth also consisted of epithelial cells only.

(b) *Carcinomatous Gland from the Neck* (secondary to carcinoma of the floor of the mouth).—Small portions of this tumour tissue grew most success-




3



fully in a medium composed of fowl plasma 1 part + extract of embryonic chick 1 part. Unlike the previous tumour, this one was somewhat tough and fibrous, due to the presence of a considerable amount of connective tissue stroma, and it is interesting to note that the new growth in this case consisted of both tissues.

After 44 hours' incubation at 37·5° C., long branching stroma cells appeared growing out from the original tissue. After five days, there appeared in several places solid buds composed of epithelial cells, and these increased in size day by day. Fig 4 represents a microphotograph of the live tissue after nine days' incubation and shows clearly the outgrowth of stroma cells and also new buds of epithelial cells of the cancer. Fig 5 shows definitely a solid outgrowth of cancerous epithelial cells after nine days' incubation, and fig. 6 shows the marked increase of the same portion after 13 days' incubation. Growth ceased after 15 days As in the case of the papilloma of the ovary the new growing epithelial cells were again much larger than the original. They were amœboid and were filled with highly refractile granules. It is interesting to note that these human tumour tissues were cultivated in a medium composed chiefly of fowl blood plasma, or, in other words, the human tissue proliferated in a nutrient material obtained entirely from a bird. This is contrary to what was previously believed, since it was considered that the tissue of a certain animal could only grow in a medium composed of the blood plasma of the same species of animal

Fuller details of this work, and parallel researches on the cultivation of the normal tissues of other animals, will be published in the Proceedings of the Royal Society of Medicine.

EXPLANATION OF PLATE.

(All the figures represent microphotographs of the live growing tissue.)

Fig. 1.—Papilloma of ovary, tissue after eight days' incubation Note the outgrowth of processes composed of epithelial cells. × 50 diameters.

Fig. 2.—Another portion of the same after nine days' incubation The medium is becoming liquefied and the new cells are somewhat scattered × 90.

Fig. 3.—Higher magnifications of the new growing cells. Note the amœboid protoplasmic processes and the highly refractile granules. × 870

Fig. 4.—Cancerous lymphatic gland, tissue after nine days' incubation. Note the buds of epithelial cancer cells and also the outgrowth of connective tissue stroma-cells. × 80

Fig. 5.—Another portion of the same, after nine days' incubation Note the solid outgrowth of epithelial cancer cells. × 80.

Fig. 6.—Same portion after thirteen days' incubation. Note the marked increase of the outgrowth of epithelial cancer cells. × 80.

Trypanosome Diseases of Domestic Animals in Nyasaland. Trypanosoma capræ (Kleine) Part III.—*Development in* Glossina morsitans.

By Surgeon-General Sir DAVID BRUCE, C B, F R.S, A.M S., Major A E HAMERTON, D S O, and Captain D P WATSON, R.A.M C, and Lady BRUCE, R.R C (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

(Received April 7,—Read June 18, 1914)

[PLATE 8]

INTRODUCTION

In a previous paper* the morphology and action on animals of this species of trypanosome were described In this is given an account of its development in *Glossina morsitans*

Trypanosoma capræ belongs to the *T vivax* group, in which the development of the trypanosomes is restricted to the proboscis

THE DEVELOPMENT OF T CAPRÆ IN G MORSITANS

Six experiments were made with laboratory-bred flies Five were positive and one negative

Table I —Laboratory-bred Flies

Date	Expt	No of flies used.	Experiment positive or negative	No of infected flies found.	No of days before flies became infective	Mean temperature
1912						
April 16	444	12	+	1	16	71° F (22 1° C)
June 8	617	38	—	0	—	65° F. (18·3° C)
„ 8	1215	22	+	1	21	65° F (18 3° C)
1913						
Jan 18	1777	35	+	11	19	84° F (28·8° C)
„ 22	1784	35	+	20	19	84° F. (28 8° C)
April 1	2046	33	+	13	20	84° F (28 8° C)

One hundred and seventy laboratory-bred flies were used and forty-six infected flies were found—27 1 per cent The first three experiments were carried out at the ordinary temperature of the laboratory, in the last three the cages containing the flies were kept in an incubator It is difficult to understand the difference in the number of infected flies found. In Experiments 444 and 1215 only 8 and 5 per cent. respectively of the flies became infected, whereas in the last three experiments, an average of more than 40 per cent. was found The flies in the second group were kept, it is

* 'Roy Soc. Proc,' B, vol. 86, p. 278 (1913).

true, at a temperature similar to that which they would find in summer in the low country, while the first three experiments were done in winter and at the ordinary temperature of the laboratory This no doubt would explain the difference to some extent Again, goats and sheep infected with *T capræ* are unsatisfactory animals to feed flies on. One day the trypanosomes are present in small numbers in the blood, the next day it may be impossible to find any, very seldom are they in any numbers It is quite possible, then, that flies may feed on an infected goat or sheep without taking in a single trypanosome

Details of the Six Experiments Five Positive, One Negative

The following table gives the principal details in carrying out the six experiments Laboratory-bred flies were used in all.

Table II

Expt.	Day of expt.	Procedure	Remarks
444	1–4 5–6 7–24	12 flies fed on infected Goat 839 Starved Fed on clean Goat 419	Trypanosomes appeared in blood of Goat 419 after 23 days All flies dissected, 1 infected fly found Goat 389 contained few trypanosomes in its blood
617	1–4 5 6–63	33 flies fed on infected Sheep 347 Starved Fed on clean Goat 628	Trypanosomes never appeared in blood of Goat 628 All flies dissected, all negative Sheep 347 was unsatisfactory, one day its blood contained a few trypanosomes, the next day none
1215	1–3 4 5–29	22 flies fed on infected Goat 979 Starved Fed on clean Goat 1219.	Trypanosomes appeared in blood of Goat 1219 after 28 days All flies dissected, 1 infected fly found
1777	1–5 6 7–27	35 flies fed on infected Goat 1746 Starved Fed on clean Goat 1803.	Trypanosomes appeared in blood of Goat 1803 after 26 days All flies dissected; 11 infected flies found
1784	1–4 5 6–27	35 flies fed on infected Goat 1746 Starved Fed on clean Goat 1812	Trypanosomes appeared in blood of Goat 1812 after 26 days All flies dissected, 20 found infected
2046	1–5 6 7–18 19–20 21–23 24–25 26–36	33 flies fed on infected Goat 1912 Starved. Fed on clean Monkey 2066 Starved Fed on clean Goat 2102 Starved Fed on clean Monkey 2066	Trypanosomes appeared in blood of Goat 2102 after 27 days All flies dissected, 18 found infected

It would appear from the five positive experiments that an average period of 19 days elapses before the cycle of development of *T caprœ* is complete in *G morsitans* and the fly becomes infective.

RESULT OF THE DISSECTION OF THE INFECTED FLIES

Table III—Laboratory-bred Flies Positive Experiments.

Expt	Time, days	Proboscis		Proventriculus	Crop	Fore-gut	Mid-gut.	Hind-gut	Salivary glands
414	25	+		–	–	–	–	–	–
1215	32	+		–	–	–	–	–	–
1777	21	+		–	–	–	–	–	–
1777	26	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1777	30	+		–	–	–	–	–	–
1784	19	+		–	–	–	–	–	–
1784	21	+		–	–	–	–	–	–
1784	23	+		–	–	–	–	–	–
1784	24	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	29	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
1784	30	+		–	–	–	–	–	–
		Labial cavity.	Hypopharynx						
2046	23	+	+	–	–	–	–	–	–
2046	23	+	–	–	–	–	–	–	–
2046	24	+	+ +	–	–	–	–	–	–
2046	26	+ +	+ +	–	–	–	–	–	–
2046	28	+		–	–	–	–	–	–
2046	28	+		–	–	–	–	–	–
2046	29	+ +	+ +	–	–	–	–	–	–
2046	29	+	+ +	–	–	–	–	–	–
2046	29	+	+ +	–	–	–	–	–	–
2046	29	+	+ +	–	–	–	–	–	–
2046	29	+ +	+ +	–	–	–	–	–	–
2046	29	+ +	+	–	–	–	–	–	–
2046	30	+	+ +	–	–	–	–	–	–

It will be seen from the above table that it was not until the last experiment that the labial cavity and hypopharynx were examined separately In the previous experiments the presence or absence of trypanosomes in the proboscis as a whole was noted

In the first two experiments, only a single infected fly was found in each. In Experiment 1777, 11, and in 1784 as many as 20 were found

In regard to the number of trypanosomes in the labial cavity, this may vary greatly Sometimes the lumen of the tube will be seen to be densely crowded, at other times a single colony will be seen For example, in Experiment 1777 the first infected fly, dissected on the 21st day, is noted to have had the lumen of the proboscis swarming with clusters of torpedo-shaped flagellates attached to the labrum by their flagellar ends, a few swimming free In the seventh infected fly, dissected on the 30th day, only three colonies, in the eighth one, and in the ninth two small colonies, are noted In the same way the hypopharynx may contain few, at other times it is seen to be densely packed with swarms of actively moving trypanosomes. In unstained specimens the difference in size and shape between the trypanosomes in the labial cavity and those in the hypopharynx is quite manifest

It may be stated here that, exceptionally, flagellates may be seen in the œsophagus, or that part of the alimentary tract anterior to the proventriculus Among the 46 flies described above, this was noted twice In the first instance they are reported as being very scanty, in the second as being active and in large numbers

But from Table III the broad fact stands out boldly—that in this species of trypanosome the development is confined to the labial cavity and hypopharynx, and does not take place in any other part of the fly

The Type of Trypanosomes found in the Infected Flies

No attempt has been made by the Commission to study the development of *T. capræ* in *G morsitans* in the earliest stages. This can only be done if a large number of laboratory-bred flies are available, and this was not the case at Kasu.

Plate 8 represents some of the developmental forms found in the labial cavity and hypopharynx of infected flies

Fig. 1 represents a torpedo-shaped organism taken from a single cluster growing near the bulb on the 19th day after the first infected feed.

Figs. 2 and 3 are similar shaped flagellates, also from a single group growing near the bulb on the 21st day

Figs. 4–10 are drawn from 24-day flies.

Figs. 11–19, 29 days Fig 19 has an encysted appearance.

Figs. 20–22, 30 days. It will be seen that most of the flagellates found in the labial cavity are crithidial in type They are generally ribbon-shaped, with well-defined nuclei and micronuclei and free flagella.

Figs 23–30 are from the hypopharynx and have been obtained, as a rule, by causing the fly to salivate on to a cover-glass. They represent the final stage in the cycle of development—the reversion to the infective or "blood form." They are smaller than those found in the blood of the vertebrate host, but resemble them closely in every other way *

Conclusions

1. *Trypanosoma capræ* is capable of passing through a cycle of development in *G. morsitans*, the flies becoming infective some 19 days after feeding on an infected animal.

2. *Trypanosoma capræ* belongs to the same group as *T vivax* and *T uniforme*, the development taking place only in the proboscis.

3. The final stage of the development takes place in the hypopharynx where the trypanosomes revert to the original "blood form" and become infective

DESCRIPTION OF PLATE

Figs. 1–3 —Common type of torpedo-shaped flagellates found attached in small single groups or clusters to the labrum, near the bulb, after 19 to 21 days.

Figs. 4–22 —Various other developmental forms found in the labial cavity in flies dissected 24 to 30 days after their first infected feed They are mostly crithidial in type.

Figs 23–30 —"Blood forms" from the hypopharynx These represent the final stage in the cycle of development

Stained Giemsa × 2000

Trypanosoma capræ
Development in Glossina morsitans

M. E. Bruce del. X 2000

The Trypanosome causing Disease in Man in Nyasaland: The Liwonde Strain Part I.—*Morphology.* Part II.—*Susceptibility of Animals.*

By Surgeon-General Sir DAVID BRUCE, C.B, F.R.S, A.M.S, Major A. E. HAMERTON, D.S.O, and Captain D. P. WATSON, R.A.M.C, and Lady BRUCE, R.R.C (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received April 7,—Read June 25, 1914.)

INTRODUCTION.

This strain was obtained in the "fly-area" of the Upper Shiré Valley, in the Liwonde district, which is situated about 100 miles south of the "Proclaimed Area."* At the time it was procured no cases of trypanosome disease in man had been reported from this district, lately, a case has been discovered at Mpimbi, in the south of the district, about 150 miles south of the "Proclaimed" or Sleeping Sickness Area.

Three dogs infected with the wild *Glossina morsitans* strains of this trypanosome were brought to Kasu, and other animals—monkeys, dogs and rats—were inoculated from them.

For purposes of description, measurement and comparison, only trypanosomes from rats were used.

I. *Morphology of the Liwonde Strain.*

A. *Living, Unstained.*

All three strains agree in being actively moving flagellates, but with little or no translatory movement.

B. *Fixed and Stained.*

The blood films were fixed, stained and measured as previously described in the 'Proceedings.'†

Before proceeding to describe the three strains in detail, it may be stated here that in general appearance, shape, position of nucleus, size of micronucleus, contents of cell, and undulating membrane, all three strains were similar, and in no way differed from the various strains of the trypanosome causing disease in man in Nyasaland which have already been described.

* "Trypanosomes Found in Wild *Glossina morsitans* and Wild Game in the 'Fly-belt' of the Upper Shiré Valley," 'Roy. Soc. Proc.,' B, vol. 88, p. 38 (1914).

† B, vol. 81, pp. 16 and 17.

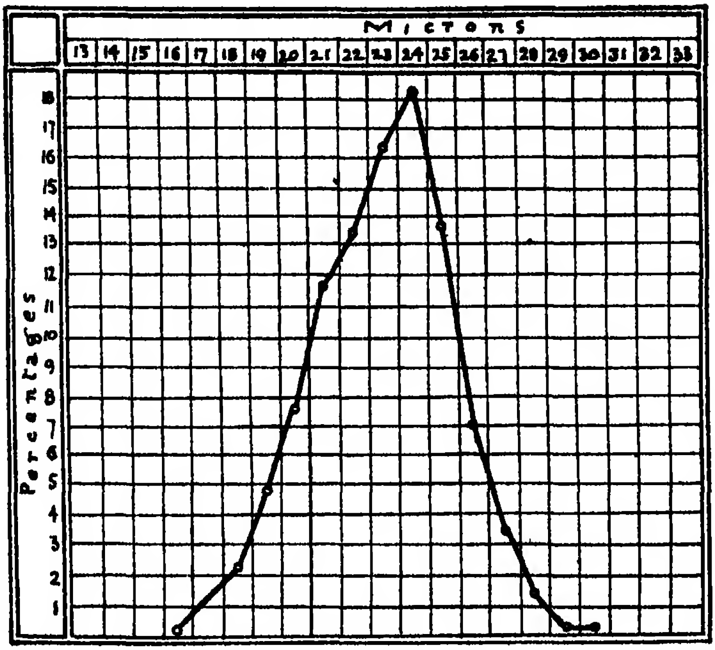
Morphology of the Liwonde Strain I

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all.

Table I.—Measurements of the Length of the Trypanosome of the Liwonde Strain I.

Date		No. of expt.	Animal	Method of fixing	Method of staining	In microns		
						Average length.	Maximum length.	Minimum length.
1918.								
Aug.	18	2378	Rat	Osmic acid	Giemsa	24·8	28·0	19·0
,,	18	2378	,,	,,	,,	24·3	27·0	21·0
,,	18	2378	,,	,,	,,	24·6	27·0	22·0
,,	19	2378	,,	,,	,,	23·4	27·0	20·0
,,	19	2378	,,	,,	,,	23·4	27·0	20·0
,,	19	2378	,,	,,	,,	24·7	27·0	21·0
,,	20	2378	,,	,,	,,	22·3	25·0	19·0
,,	20	2378	,,	,,	,,	21·4	25·0	18·0
,,	20	2378	,,	,,	,,	21·3	24·0	18·0
,,	21	2378	,,	,,	,,	22·1	26·0	16·0
,,	21	2378	,,	,,	,,	22·1	26·0	19·0
,,	21	2378	,,	,,	,,	21·2	25·0	18·0
,,	22	2378	,,	,,	,,	23·3	28·0	20·0
,,	22	2378	,,	,,	,,	23·1	26·0	20·0
,,	22	2378	,,	,,	,,	23·2	28·0	20·0
,,	23	2378	,,	,,	,,	23·3	28·0	21·0
,,	23	2378	,,	,,	,,	24·0	28·0	20·0
,,	23	2378	,,	,,	,,	24·2	30·0	20·0
,,	24	2378	,,	,,	,,	23·0	28·0	20·0
,,	24	2378	,,	,,	,,	23·1	29·0	19·0
,,	24	2378	,,	,,	,,	22·9	25·0	19·0
,,	25	2378	,,	,,	,,	22·5	27·0	18·0
,,	25	2378	,,	,,	,,	22·0	26·0	18·0
,,	25	2378	,,	,,	,,	22·0	25·0	18·0
,,	26	2378	,,	,,	,,	21·8	24·0	18·0
						23·0	30·0	16·0

CHART 1—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain I, taken on Nine consecutive Days from Rat 2378



This curve differs from the ordinary Wild *G morsitans* curve, but is similar to Strains II, IV and V of the Human strain *

Breadth—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all

Table II—Measurements of the Breadth of the Trypanosome of the Liwonde Strain I, measured across the Widest Part, including the Undulating Membrane.

Experiment No.	Animal	Number measured	In microns		
			Average breadth	Maximum breadth	Minimum breadth
2378	Rat	500	3 0	4 50	1 25

* 'Roy. Soc. Proc.,' B, vol. 86, pp 288, 295, and 298 (1913)

Table III —Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain I

Date	Experiment No	Animal	Percentage among short and stumpy forms
1913			
Aug 18	2378	Rat	2
„ 19	2378	„	23
„ 20	2378	„	17
„ 21	2378	„	4
„ 22	2378	„	12
„ 23	2378	„	16
„ 24	2378	„	28
„ 25	2378	„	15
„ 26	2378	„	19
„ 27	2378	„	22
		Average	15 8

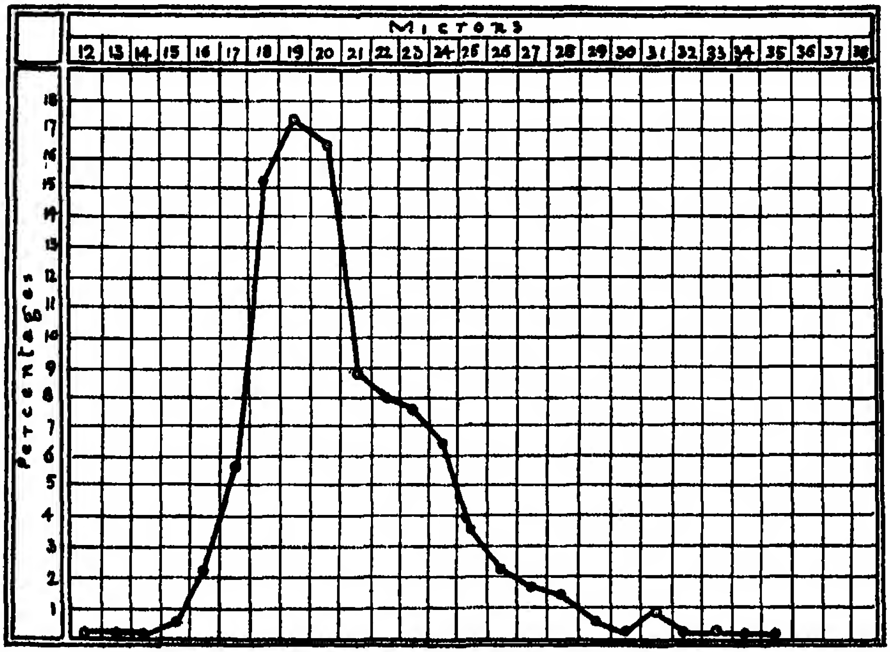
Morphology of the Liwonde Strain II

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all —

Table IV —Measurements of the Length of the Trypanosome of the Liwonde Strain II.

Date	No of expt	Animal	Method of fixing	Method of staining.	In microns		
					Average length	Maximum length	Minimum length
1913							
Aug 18	2363	Rat	Osmic acid	Giemsa	20 ·3	31 0	17 ·0
„ 18	2363	„	„	„	19 ·0	23 0	14 ·0
„ 18	2363	„	„	„	20 8	26 ·0	18 ·0
„ 19	2363	„	„	„	19 2	25 0	18 ·0
„ 19	2363	„	„	„	20 6	25 ·0	17 ·0
„ 19	2363	„	„	„	19 7	24 ·0	17 ·0
„ 20	2363	„	„	„	20 7	26 ·0	18 ·0
„ 20	2363	„	„	„	21 2	29 ·0	18 ·0
„ 20	2363	„	„	„	20 ·0	24 ·0	17 ·0
„ 21	2363	„	„	„	19 ·0	24 0	16 ·0
„ 21	2363	„	„	„	19 9	28 ·0	18 ·0
„ 21	2363	„	„	„	19 8	26 ·0	17 0
„ 22	2363	„	„	„	21 ·9	27 ·0	18 0
„ 22	2363	„	„	„	21 ·9	28 ·0	18 ·0
„ 22	2363	„	„	„	22 ·9	27 0	20 ·0
„ 23	2363	„	„	„	22 ·6	33 ·0	18 ·0
„ 23	2363	„	„	„	22 9	34 ·0	18 ·0
„ 23	2363	„	„	„	21 ·0	30 0	17 ·0
„ 24	2363	„	„	„	20 9	35 ·0	17 0
„ 24	2363	„	„	„	22 2	31 ·0	16 0
„ 24	2363	„	„	„	20 ·1	31 0	16 ·0
„ 25	2363	„	„	„	20 9	28 ·0	16 ·0
„ 25	2363	„	„	„	20 5	28 ·0	15 ·0
„ 25	2363	„	„	„	20 6	27 ·0	12 ·0
„ 26	2363	„	„	„	20 1	31 ·0	17 ·0
					20 7	35 ·0	12 ·0

CHART 2.—Curve representing the Distribution by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain II, taken on Nine consecutive Days, from Rat 2363.



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all:—

Table V.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain II, measured across the Widest Part, including the Undulating Membrane.

Experiment No.	Animal	Number measured	In microns		
			Average breadth	Maximum breadth	Minimum breadth
2363	Rat	500	3·0	4·75	1·25

Table VI.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain II.

Date	Experiment No.	Animal	Percentage among short and stumpy forms
1913			
Aug. 18	2363	Rat	25
„ 19	2363	„	13
„ 20	2363	„	14
„ 21	2363	„	16
„ 22	2363	„	12
„ 23	2363	„	23
„ 24	2363	„	35
„ 25	2363	„	37
„ 26	2363	„	14
„ 27	2363	„	23
		Average	21·2

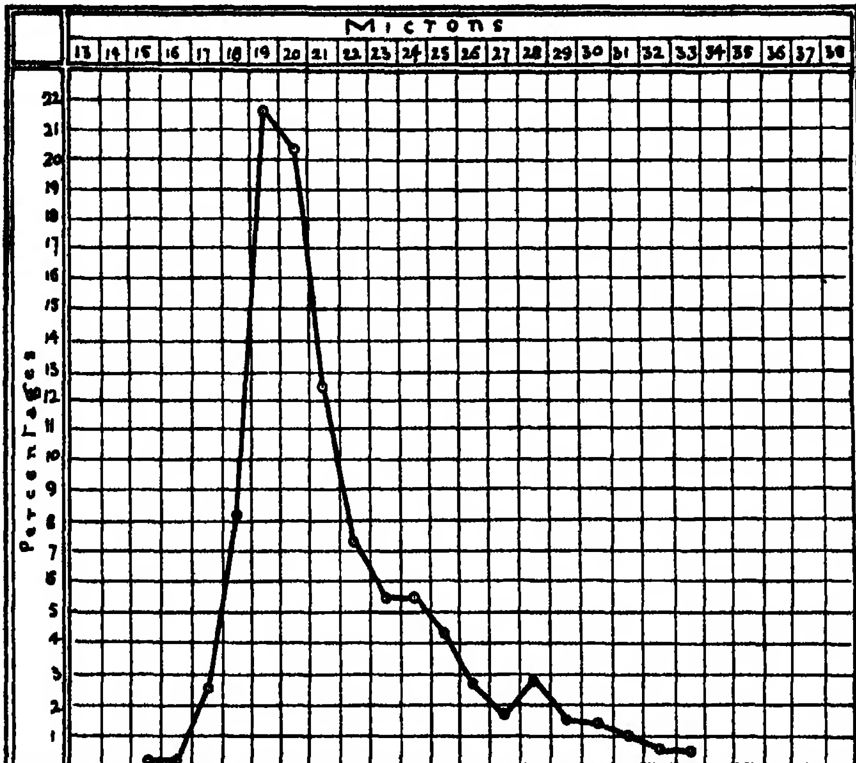
Morphology of the Liwonde Strain III.

Length.—The following table gives the length of this trypanosome as found in the white rat—500 trypanosomes in all:—

Table VII.—Measurements of the Length of the Trypanosome of the Liwonde Strain III.

Date	No. of expt.	Animal	Method of fixing	Method of staining	In microns: Average length.	Maximum length.	Minimum length.
1913							
Aug. 18	2370	Rat	Osmic acid	Giemsa	22·9	29·0	18·0
„ 18	2370	„	„	„	22·1	30·0	17·0
„ 18	2370	„	„	„	22·6	31·0	18·0
„ 19	2370	„	„	„	19·7	24·0	18·0
„ 19	2370	„	„	„	19·8	23·0	18·0
„ 19	2370	„	„	„	19·9	28·0	18·0
„ 20	2370	„	„	„	20·9	25·0	19·0
„ 20	2370	„	„	„	21·2	30·0	18·0
„ 20	2370	„	„	„	22·6	32·0	19·0
„ 21	2370	„	„	„	20·0	24·0	17·0
„ 21	2370	„	„	„	21·0	28·0	17·0
„ 21	2370	„	„	„	20·8	28·0	17·0
„ 22	2370	„	„	„	19·9	29·0	17·0
„ 22	2370	„	„	„	20·1	25·0	15·0
„ 22	2370	„	„	„	20·5	29·0	17·0
„ 23	2370	„	„	„	23·7	30·0	19·0
„ 23	2370	„	„	„	23·9	32·0	18·0
„ 23	2370	„	„	„	23·0	30·0	17·0
„ 24	2370	„	„	„	21·7	33·0	17·0
„ 24	2370	„	„	„	23·0	33·0	18·0
„ 24	2370	„	„	„	22·2	27·0	18·0
„ 25	2370	„	„	„	21·7	31·0	18·0
„ 25	2370	„	„	„	21·0	30·0	18·0
„ 25	2370	„	„	„	20·9	29·0	17·0
„ 26	2370	„	„	„	19·7	26·0	16·0
					21·4	33·0	15·0

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Liwonde Strain III, taken on Nine consecutive Days, from Rat 2370



Breadth.—The following table gives the breadth of this trypanosome in the rat—500 trypanosomes in all :—

Table VIII.—Measurements of the Breadth of the Trypanosome of the Liwonde Strain III, measured across the Widest Part, including the Undulating Membrane.

Experiment No	Animal	Number measured.	In microns		
			Average breadth	Maximum breadth.	Minimum breadth
2370	Rat	500	2·6	4·75	1·25

Table IX.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Liwonde Strain III.

Date	Experiment No.	Animal	Percentage among short and stumpy forms
1913			
Aug. 18	2370	Rat	4
„ 19	2370	„	44
„ 20	2370	„	36
„ 21	2370	„	33
„ 22	2379	„	22
„ 23	2370	„	4
„ 24	2378	„	6
„ 25	2370	„	12
„ 26	2370	„	30
„ 27	2370	„	33
		Average	22 4

COMPARISON OF THE LIWONDE STRAINS WITH ONE ANOTHER.

Table X.—Measurements of the Length of the Trypanosome of the Liwonde Strains.

Date	Experiment No.	Strain	Animal	Number of trypanosomes measured	In microns		
					Average length	Maximum length	Minimum length
1913	2378	I	Rat	500	23 0	30·0	16·0
1913	2368	II	Rat	500	20 7	35·0	12·0
1913	2370	III	Rat	500	21 4	33 0	15 0
					21 7	35·0	12 0

CHART 4 —Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of the Liwonde Strains.

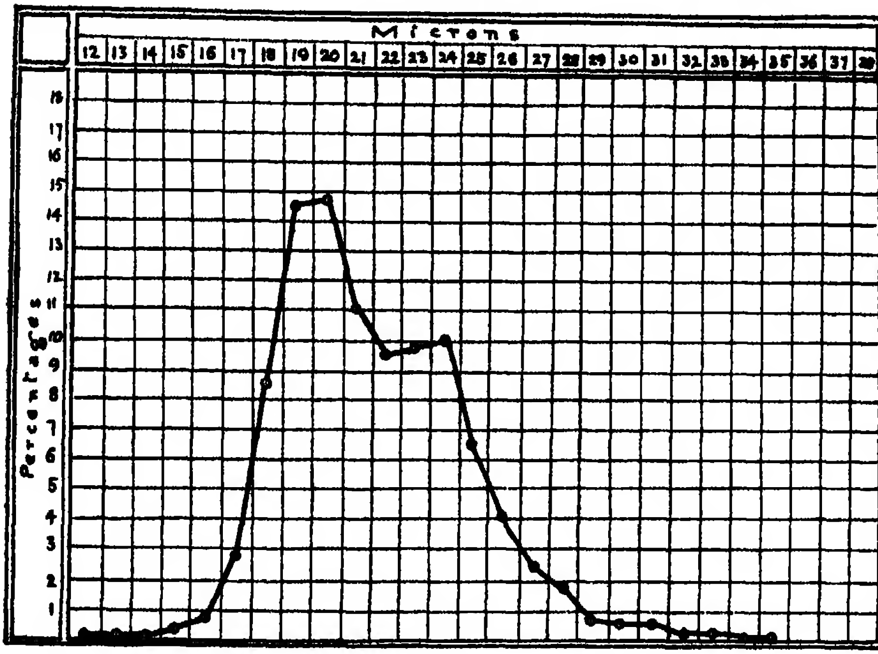


Table XI —Measurements of the Breadth of the Trypanosome of the Liwonde Strains

Date	Experiment No	Strain.	Animal	Number of trypanosomes measured	In microns		
					Average breadth	Maximum breadth	Minimum breadth
1913	2378	I	Rat	500	3·0	4 50	1 25
1913	2368	II	Rat	500	3 0	4·75	1 25
1913	2370	III	Rat	500	2 6	4 75	1 25
					2 9	4·75	1 25

Table XII.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Three Liwonde Strains

Date	Experiment No	Strain	Animal	Percentage among short and stumpy forms
1913	2378	I	Rat	15 8
1913	2363	II	"	21 2
1913	2370	III	"	22 4
			Average	19·8

COMPARISON OF THE LIWONDE STRAIN WITH THE HUMAN, WILD-GAME, AND WILD GLOSSINA MORSITANS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND

Table XIII.—Average Length of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains

Strain	Number of trypanosomes measured	Animal	In microns		
			Average length	Maximum length	Minimum length
Human	5500	Rat	23 5	38·0	14·0
Wild-game	2500	"	22·6	35·0	15·0
Wild *G morsitans*	2500	"	22·6	35 0	15 0
Liwonde	1500	"	21·7	35 0	12·0
			22·6	38 0	12 0

CHART 5.—Curve representing the Distribution, by Percentages, in respect to Length, of 1500 Individuals of the Trypanosome of the Liwonde Strain, 5000 of the Wild-game and Wild *Glossina morsitans* combined, and 5500 of the Human Strain, all measured from Rats

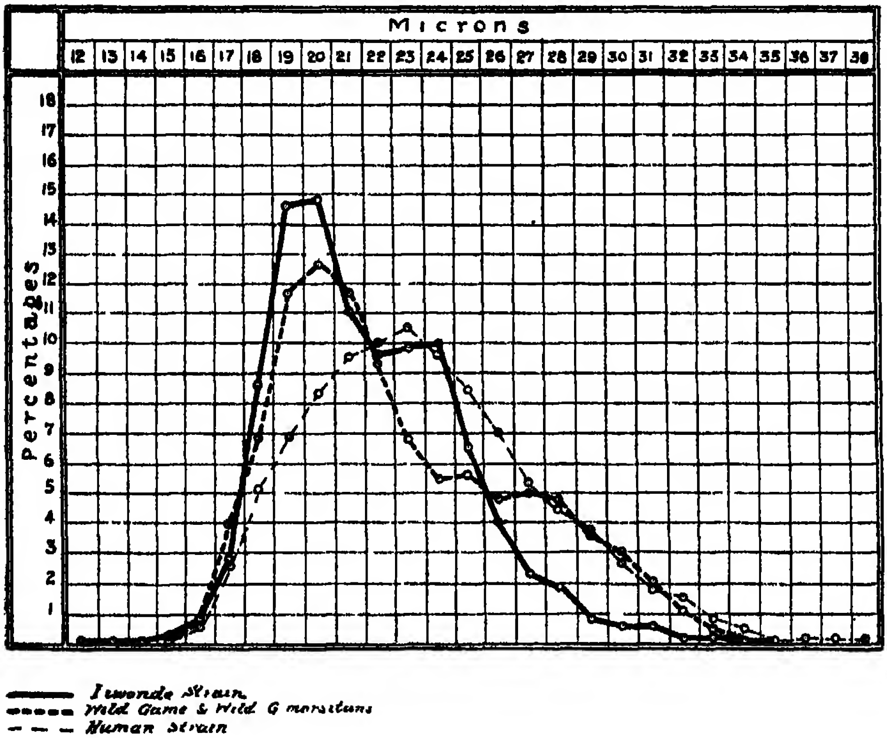


Table XIV.—Average Breadth of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains

Strain	Number of trypanosomes measured.	Animal	In microns.		
			Average breadth	Maximum breadth	Minimum breadth
Human	1500	Rat	2 6	5 0	1 25
Wild-game	1500	"	3 2	5 75	1 50
Wild *G morsitans*	1500	"	2·9	5 25	1 25
Liwonde	1500	"	2·9	4 75	1 25
			2 9	5 75	1·25

Table XV.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Liwonde Strains.

Date	Strain	Animal	Percentage among short and stumpy forms
1912	Human	Rat	17 8
1912	Wild-game	„	26 2
1912	Wild *G morsitans*	„	12 5
1913	Liwonde	„	19 8
		Average	19 1

II. *Animals susceptible to the Trypanosome of the Liwonde Strain.*

The following tables give the incubation and duration of the disease in goats, monkeys, dogs, guinea-pigs, and white rats, which were sub-inoculated with Strains I, II, and III from dogs infected in the Liwonde district:—

Liwonde Strain I

Table I

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Goat		
1913 Aug. 12	2382	Monkey 2340	6	52	Died of Strain I
„ 12	2383	„ 2340	6	44	„ „
			Monkey.		
Aug 7	2339	Dog 2322	4	27	Died of Strain I
„ 7	2339	„ 2322	4	72	„ „
			Dog		
July 16	2322	Wild flies	6	23	Died of Strain I
Aug 12	2380	Monkey 2340	6	46	„ „
„ 12	2381	„ 2340	6	45	„ „
			Guinea-pig		
Aug 12	2384	Monkey 2340	18	78	Died of Strain I
„ 12	2385	„ 2340	23	114	„ „
			Rat		
Aug. 12	2378	Monkey 2340	6	85	Died of Strain I.
„ 12	2379	„ 2340	6	44	„ „

* Duration includes the days of incubation; it dates from day of inoculation.

Liwonde Strain II.

Table II

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Goat		
1913					
Aug 12	2366	Monkey 2336	6	39	Died of Strain II
" 12	2367	" 2336	9	41	" "
			Monkey.		
Aug 6	2335	Dog 2323	5	32	Died of Strain II
" 6	2336	" 2323	5	43	" "
			Dog		
July 5	2353	Wild flies	3	—	Killed July 28
" 28	2323	Dog 2353	9	17	Died of Strain II
Aug 12	2364	Monkey 2336	6	23	" "
" 12	2365	" 2336	6	27	" "
			Guinea-pig		
Aug 12	2368	Monkey 2336	40	94	Died of Strain II
" 12	2369	" 2336	—	—	Never showed trypanosomes
			Rat		
Aug 12	2391	Monkey 2336	5	22	Died of Strain II
" 12	2363	" 2336	6	21	" "

Liwonde Strain III

Table III

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Goat		
1913					
Aug 12	2374	Monkey 2338	6	10	Died of Strain III
" 12	2375	" 2338	9	12	" "
			Monkey		
Aug 6	2337	Dog 2321	5	32	Died of Strain III
" 6	2338	" 2321	5	38	" "
			Dog		
July 8	2356	Wild flies	4	—	Killed July 28
" 28	2321	Dog 2356	9	16	Died of Strain III
Aug. 12	2372	Monkey 2338	6	39	" "
" 12	2373	" 2338	6	12	" "
			Guinea-pig.		
Aug 12	2376	Monkey 2338	20	39	Died of Strain III
" 12	2377	" 2338	73	93	" "
			Rat		
Aug 12	2370	Monkey 2338	6	20	Died of Strain III.
" 12	2371	" 2338	6	17	" "

* Duration includes the days of incubation; it dates from day of inoculation

Disease Set Up in Various Animals by the Trypanosome of the Liwonde Strain

The infection set up in the various animals by the Liwonde strain gave rise to symptoms and appearances during life, and pathological changes in the various organs after death, alike and similar in every way to those caused by the Human strain, Wild-game strain, and the Wild *G morsitans* strain of *T. brucei vel rhodesiense* found in the "Proclaimed Area"

COMPARISON OF THE THREE LIWONDE STRAINS IN REGARD TO THEIR VIRULENCE TOWARDS VARIOUS ANIMALS

Table IV—The Average Duration, in Days, of the Disease in various Animals

Strain	Goat	Monkey	Dog	Guinea pig	White rat
I	48	49	38	96	39
II	40	37	22	94	21
III	11	35	22	66	18

No recoveries took place among the experimental animals

Table V—The Average Duration of Life, in Days, of various Animals infected with the Liwonde Strain

	Goat	Monkey	Dog	Guinea-pig,	White rat.
Average duration, in days	33	41	28	84	26
Number of animals employed	6	6	9	5	6

COMPARISON OF THE LIWONDE STRAIN WITH THE HUMAN STRAIN

Table VI.—The Average Duration of Life, in Days, of various Animals infected with the Human and the Liwonde Strains The letter R stands for "refractory"

	Strain.	Ox	Goat and sheep	Baboon	Monkey	Dog	Rabbit.	Guinea-pig	White rat
Average duration, in days	Human	184	42	R	26	34	28	67	30
Average duration, in days	Liwonde	—	33	—	41	28	—	84	26

CONCLUSIONS.

1 The three wild *G morsitans* strains from the Liwonde district resemble each other closely, and all belong to the same species of trypanosome

2 The Liwonde strain belongs to the same species as that occurring in man, wild game, and wild *G morsitans* inhabiting the "Proclaimed Area," Nyasaland—*T brucei vel rhodesiense*

3. Hence it would appear that wild *G. morsitans* occurring in a district 100 miles south of the "Proclaimed Area" are infected with the trypanosome which causes the human trypanosome disease of Nyasaland

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The Trypanosome causing Disease in Man in Nyasaland The Naturally Infected Dog Strain Part I.—*Morphology.*

By Surgeon-General Sir DAVID BRUCE, C B, F R S, A M S, Major A E HAMERTON, D S O, and Captain D P WATSON, R A M C, and Lady BRUCE, R R C (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

(Received April 7,—Read June 25, 1914)

[PLATES 9–11]

INTRODUCTION

This strain differs so much from the others that it is doubtful if it should be included among the various strains already described, Human,* Wild-game,† Wild *Glossina morsitans*,‡ Mzimba,§ etc It has only been found on three occasions and, curiously enough, each time in a native dog

The three dogs suffering from trypanosome disease were brought up to Kasu from the "Proclaimed Area," where they had probably been naturally infected by the wild *G morsitans*, hence the name "The Naturally Infected Dog Strain."

All the infected dogs coming from this area did not show this strain, for example, Dog 553 was infected with a trypanosome resembling the ordinary Human strain

* 'Roy Soc. Proc.,' B, vol. 85, p. 423 (1912), and vol. 86, p 285 (1913).

† *Ibid*, B, vol. 86, p. 394 (1913).

‡ *Ibid*, B, vol. 86, p. 408 (1913)

§ *Ibid*, B, vol 87, p. 26 (1913).

If this Naturally Infected Dog strain had been found in the blood of the wild game and in the wild *G. morsitans*, then it would have been legitimate to make a new species of it. But it would be unjustifiable to make a new species of a strain which, up to the present, has only been found in three chronically infected dogs. The Commission have therefore decided to consider this strain as belonging to the species described as the Trypanosome causing Disease in Man in Nyasaland—*Trypanosoma brucei vel rhodesiense*—and not as a new species. If this is correct, then it is curious how much a species can vary in disease-producing power. For example, it will be shown that this Naturally Infected Dog strain is almost harmless to monkeys and guinea-pigs, whereas the parent species kills these animals without fail. Not only does it differ in virulence, but even its morphology is apparently somewhat changed. There is a comparative absence of the blunt-ended posterior-nucleated forms, which are sometimes so marked a feature in the parent species. Not that they are altogether absent, but they are not so prominent, do not strike the eye so readily. It will therefore be interesting to describe this strain as fully and completely as possible.

MORPHOLOGY OF THE NATURALLY INFECTED DOG STRAIN STRAIN I. DOG 48

Table I—Measurements of the Length of the Trypanosome of Naturally Infected Dog. Strain I. Dog 48

Date	No of expt	Animal	Method of fixing	Method of staining	In microns		
					Average length	Maximum length	Minimum length
1912							
Feb 17	191	Ox	Osmic acid	Giemsa	25 5	31·0	16 0
" 22	196	Sheep	"	"	26 4	30 0	21·0
Jan 20	48	Dog	"	"	23 6	29 0	19 0
" 24	48	"	"	"	24 2	31 0	16·0
Feb 1	48	"	"	"	25 7	35·0	15 0
" 15	140	"	"	"	24 5	30 0	17 0
" 19	139	"	"	"	29 8	38 0	20·0
" 19	139	"	"	"	32·3	35 0	25 0
" 22	69	"	"	"	30 1	33 0	26·0
" 26	151	"	"	"	28 5	32·0	23·0
" 28	210	"	"	"	22 8	32·0	19·0
" 29	210	"	"	"	20 1	23·0	16·0
April 15	317	"	"	"	20 1	31 0	18 0
" 19	317	"	"	"	23 2	31·0	19·0
" 22	331	"	"	"	22·6	31 0	16·0
" 8	389	Rabbit	"	"	22 9	29·0	15·0
" 15	389	"	"	"	26·0	29·0	18·0
" 15	390	"	"	"	22 3	35·0	15·0
" 16	389	"	"	"	24 3	30 0	17·0
Feb 13	67	Rat	"	"	21 4	28 0	18 0
" 13	67	"	"	"	20·9	31·0	17·0
" 13	67	"	"	"	22 8	31·0	18·0
" 15	67	"	"	"	19 8	30 0	17·0
" 15	189	"	"	"	24 9	30·0	18 0
" 15	190	"	"	"	23 6	29·0	17·0
" 19	189	"	"	"	28 6	35·0	21 0
" 22	190	"	"	"	28·0	34 0	18·0
" 26	67	"	"	"	23 2	33 0	18·0
" 29	67	"	"	"	20 5	31 0	16·0
Mar 11	67	"	"	"	20 7	30·0	16 0
April 3	312	"	"	"	26 6	32 0	18·0
" 5	312	"	"	"	21 7	32 0	18 0
" 8	391	"	"	"	20 3	30 0	16 0
" 8	392	"	"	"	24 8	32 0	16 0
" 8	392	"	"	"	25 8	32·0	17·0
" 8	392	"	"	"	25 5	32 0	16 0
April 11	311	"	"	"	29 5	35 0	19 0
" 11	312	"	"	"	25 4	32 0	16 0
" 12	311	"	"	"	20 8	31 0	15 0
" 12	312	"	"	"	23 0	36 0	19·0
" 12	391	"	"	"	27 6	32 0	19·0
" 12	392	"	"	"	24 3	30 0	18 0
" 13	311	"	"	"	21 9	32 0	18 0
" 13	312	"	"	"	21 7	33 0	19·0
" 13	391	"	"	"	29 3	35 0	19·0
" 13	392	"	"	"	23 7	36 0	18·0
" 14	407	"	"	"	23 0	33 0	16·0
" 15	312	"	"	"	22 1	33 0	18 0
" 15	391	"	"	"	25·0	33·0	19 0
" 15	392	"	"	"	21 8	35 0	19 0
" 16	312	"	"	"	21 8	32·0	17 0
" 16	391	"	"	"	19 7	28 0	17·0
					24·2	36 0	15·0

Table II.—Measurements of the Length of 500 Specimens of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken on nine consecutive days, from Rat 1218, after passage through rats for seven months.

Date	No. of expt.	Animal	Method of fixing	Method of staining	In microns: Average length	Maximum length	Minimum length
1913							
Sept. 5	1218	Rat	Osmic acid	Giemsa	25·8	31·0	17·0
„ 5	1218	„	„	„	27·3	33·0	19·0
„ 5	1218	„	„	„	25·1	31·0	18·0
„ 6	1218	„	„	„	23·0	31·0	16·0
„ 6	1218	„	„	„	21·7	32·0	16·0
„ 6	1218	„	„	„	23·7	31·0	18·0
„ 7	1218	„	„	„	26·4	30·0	21·0
„ 7	1218	„	„	„	26·0	30·0	20·0
„ 7	1218	„	„	„	25·2	31·0	18·0
„ 8	1218	„	„	„	26·2	32·0	21·0
„ 8	1218	„	„	„	25·3	31·0	18·0
„ 8	1218	„	„	„	26·0	30·0	19·0
„ 9	1218	„	„	„	28·1	32·0	24·0
„ 9	1218	„	„	„	27·8	32·0	21·0
„ 9	1218	„	„	„	27·0	31·0	21·0
„ 10	1218	„	„	„	22·6	31·0	17·0
„ 10	1218	„	„	„	20·8	30·0	16·0
„ 10	1218	„	„	„	22·9	32·0	18·0
„ 11	1218	„	„	„	21·3	29·0	18·0
„ 11	1218	„	„	„	20·4	28·0	17·0
„ 11	1218	„	„	„	20·9	26·0	18·0
„ 12	1218	„	„	„	23·0	33·0	18·0
„ 12	1218	„	„	„	20·1	28·0	17·0
„ 12	1218	„	„	„	23·2	32·0	16·0
„ 13	1218	„	„	„	21·9	29·0	19·0
					24·1	33·0	16·0

Table III.—Measurements of the Length of 500 Specimens of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken on nine consecutive days from Rat 2471, after passage through rats for two years. Series of 46 animals.

Date	No. of expt.	Animal	Method of fixing	Method of staining	In microns		
					Average length	Maximum length	Minimum length.
1913							
Dec. 26	2471	Rat	Osmic acid	Giemsa	29·5	34·0	22·0
" 26	2471	"	"	"	28·6	32·0	25·0
" 26	2471	"	"	"	29·1	33·0	25·0
" 27	2471	"	"	"	28·4	31·0	26·0
" 27	2471	"	"	"	29·0	31·0	26·0
" 27	2471	"	"	"	28·0	33·0	19·0
" 28	2471	"	"	"	30·3	35·0	20·0
" 28	2471	"	"	"	31·0	35·0	27·0
" 28	2471	"	"	"	29·8	36·0	22·0
1914							
Jan. 1	2471	"	"	"	28·7	32·0	24·0
" 1	2471	"	"	"	28·7	31·0	25·0
" 1	2471	"	"	"	27·8	30·0	19·0
" 3	2471	"	"	"	29·3	33·0	23·0
" 3	2471	"	"	"	30·4	34·0	25·0
" 3	2471	"	"	"	30·4	34·0	26·0
" 4	2471	"	"	"	26·8	33·0	20·0
" 4	2471	"	"	"	27·6	32·0	19·0
" 4	2471	"	"	"	27·9	32·0	19·0
" 5	2471	"	"	"	28·2	33·0	20·0
" 5	2471	"	"	"	26·6	32·0	18·0
" 5	2471	"	"	"	27·3	33·0	19·0
" 6	2471	"	"	"	27·9	32·0	19·0
" 6	2471	"	"	"	28·0	33·0	24·0
" 6	2471	"	"	"	28·7	33·0	20·0
" 7	2471	"	"	"	26·9	32·0	19·0
					28·6	36·0	18·0

The average length of the trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken from Tables I, II, and III, is as follows:—

Table IV.—Average Length of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48.

Species of animal	Number of trypanosomes measured	In microns		
		Average length	Maximum length	Minimum length
Ox	20	25·5	31·0	16·0
Sheep	20	26·4	30·0	21·0
Dog	260	25·2	35·0	15·0
Rabbit	80	23·9	35·0	15·0
Rat	660	28·7	36·0	15·0

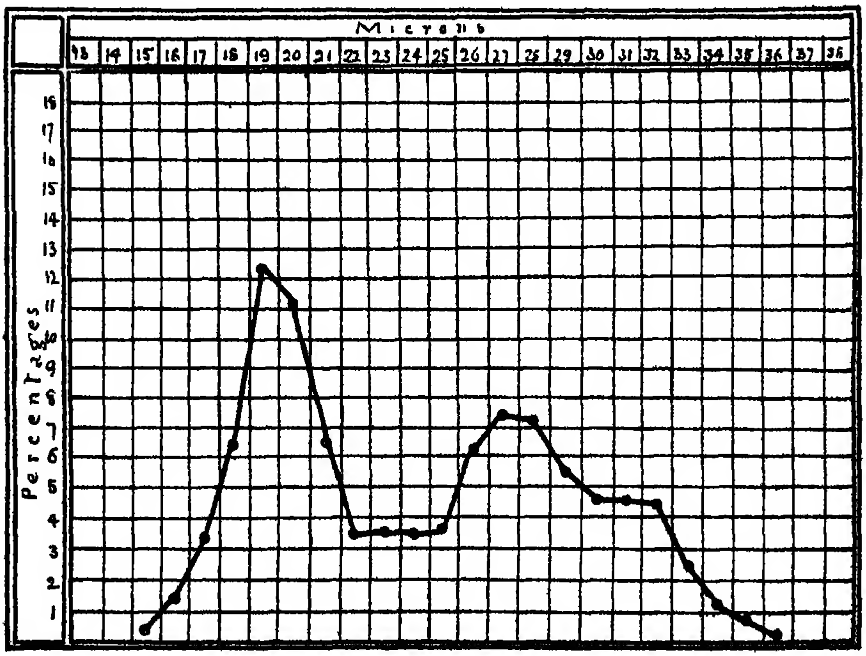
Table V.—Average Length of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, after passage through rats for seven months.

Species of animal	Number of trypanosomes measured	In microns		
		Average length	Maximum length	Minimum length
Rat	500	24 1	33 0	19·0

Table VI.—Average Length of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, after passage through rats for two years. Series of 46 animals.

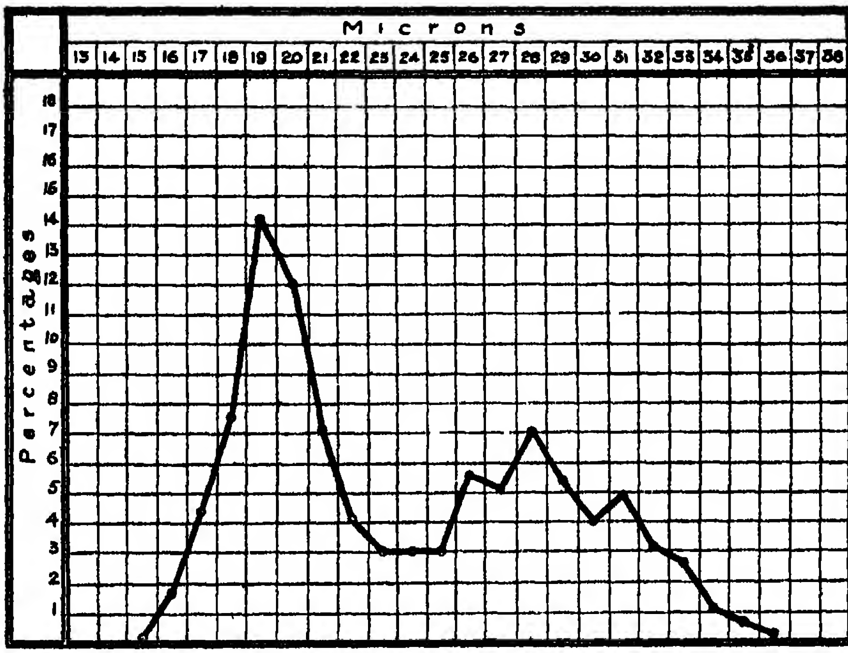
Species of animal	Number of trypanosomes measured	In microns		
		Average length	Maximum length	Minimum length
Rat	500	28 6	36 0	18·0

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 1040 Individuals of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken at random from various animals.



This curve is made up of measurements from 20 specimens of trypanosomes taken from the ox, 20 from the sheep, 260 from the dog, 80 from the rabbit, and 660 from the rat

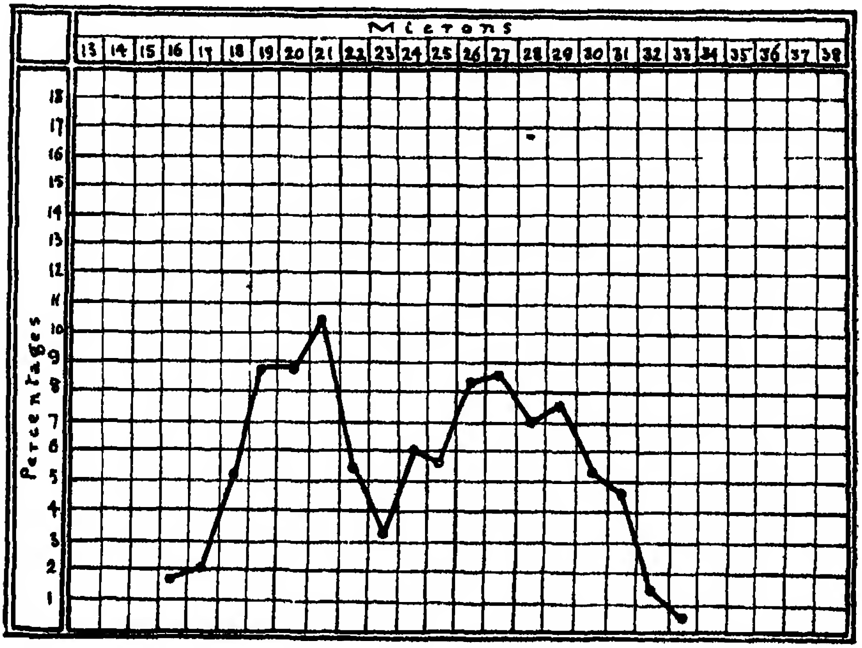
CHART 2 —Curve representing the Distribution, by Percentages, in respect to Length, of 660 Individuals of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken at random from several rats



This is the curve of a markedly dimorphic type, and may be compared with the Wild-game strain I,* or with the Wild *G morsitans* strains IV and V.† The above curve shows the strain as it appeared in the rat in February, 1912, when it was first obtained The next curve shows the same strain as it appeared in the rat in September, 1912, after it had passed for seven months through a series of eight rats

* 'Roy Soc Proc,' B, vol. 87, p. 395 (1913)
† *Ibid.*, B, vol. 87, pp. 415 and 417 (1913).

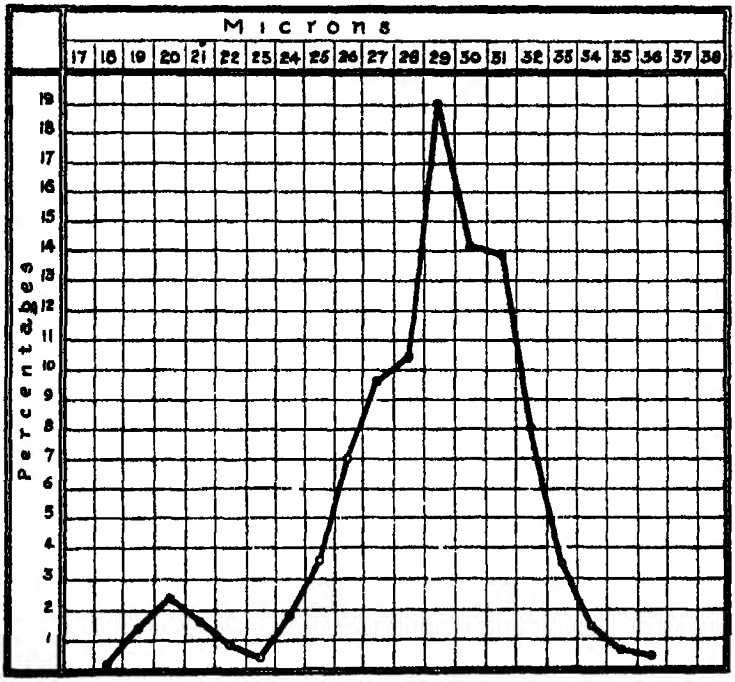
CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken on nine consecutive days from Rat 1218, after passing through a series of eight rats



This curve still shows a markedly dimorphic type, but the proportion of the long forms is increasing.

The next curve shows the same strain at the beginning of 1914, after passing for two years through a series of 46 rats.

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken on nine consecutive days from Rat 2471, after passage through rats for two years

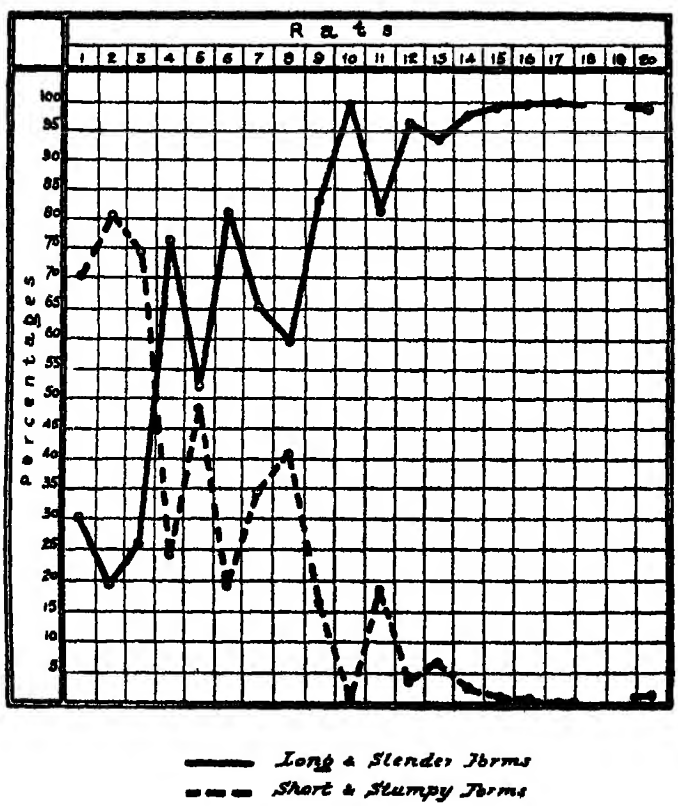


The curve is now practically monomorphic The short and stumpy and the intermediate forms have almost disappeared, and only the long and slender survive. But, it may be objected, perhaps this curve from Rat 2471 is a mere accident due to some peculiarity in the rat, it is possible that if another rat is inoculated from it the curve will be found to be as dimorphic in type as that of Rat 1218 on Chart 3 That this is not so will be seen by the following chart, which represents the gradual change in type which takes place in this trypanosome by passage through rats The first rat, 67, was inoculated from the original dog; the second rat, 312, was inoculated from Rat 67, Rat 407 from Rat 312, and so on through a series of 20 rats The unbroken line represents the percentage of the long and slender forms, the broken line the short and stumpy For example, Rat 67 has 30 per cent. long and slender and 70 per cent. short and stumpy In Rat 670 the long and short forms are almost equally divided, Rat 786, 82 per cent. long and 18 per cent. short. The percentage of the long and slender gradually increases until at the end of 17 passages it reaches 100 per cent., so that

from a dimorphic type with 70 per cent short forms the type gradually changes into a monomorphic type which has lost almost all the short forms and nothing but the long remain

This seems to show how fallacious it is to reason from laboratory types of trypanosomes to the wild natural types, and probably accounts for the showers of new species which are constantly falling about our ears.

CHART 5.—Curves representing the Gradual Change of this Trypanosome from a Dimorphic Type to a Monomorphic.



Breadth.—The following table gives the breadth of Strain I, Dog 48 :—

Table VII.—Measurements of the Breadth of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48.

Date	Experiment No	Animal	Number of trypanosomes measured.	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1912	1218	Rat	500	2 90	5·00	1 25

Table VIII.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48.

Date	Experiment No	Animal	Percentage among short and stumpy forms
1912			
April 22	407	Rat	3
" 25	407	"	2
" 29	407	"	1
May 2	407	"	4
" 6	407	"	3
" 9	407	"	8
" 13	407	"	6
" 16	407	"	8
" 20	407	"	4
" 23	407	"	23
" 27	407	"	16
		Average	7 0

Table IX.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, after passage through rats for seven months.

Date	Experiment No	Animal	Percentage among short and stumpy forms
1912.			
Sept. 5	1218	Rat	5
" 6	1218	"	3
" 7	1218	"	4
" 8	1218	"	0
" 9	1218	"	0
" 10	1218	"	0
" 11	1218	"	0
" 12	1218	"	0
" 13	1218	"	1
" 14	1218	"	0
		Average .	1 3

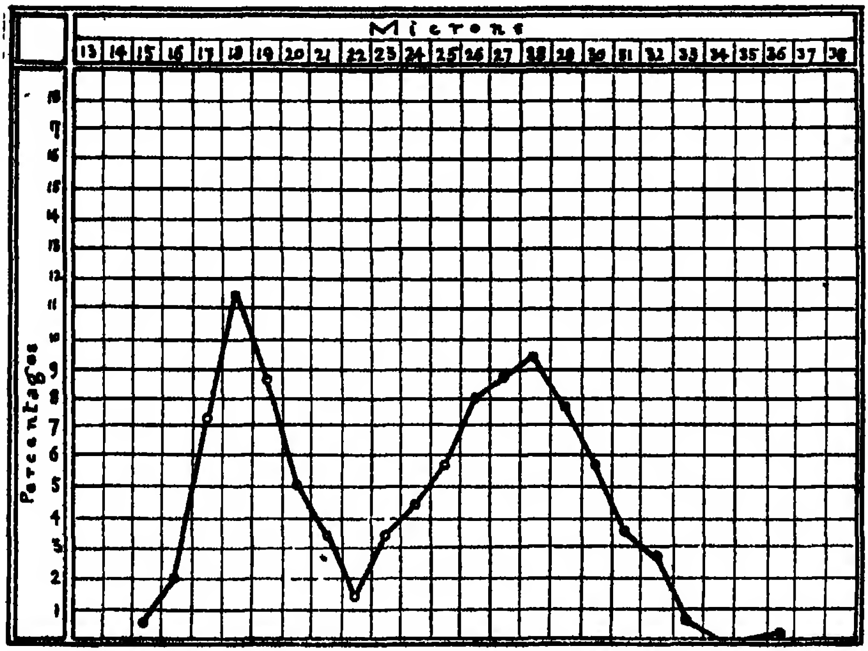
After passage through rats for two years the short and stumpy forms have nearly all disappeared, and with them the posterior-nucleated forms. In Rat 407, on Table VIII, there are a fair number of posterior-nucleated trypanosomes, on one day as many as 23 per cent, but this is exceptional.

MORPHOLOGY OF THE NATURALLY INFECTED DOG STRAIN. STRAIN II. DOG 690.

Table X.—Measurements of the Length of the Trypanosome of Naturally Infected Dog, Strain II, Dog 690.

Date	No. of expt.	Animal	Method of fixing	Method of staining	In microns: Average length	In microns: Maximum length.	In microns: Minimum length
1912							
July 26	911	Rat	Osmic acid	Giemsa	25·3	32·0	18·0
" 26	911	"	"	"	23·6	30·0	18·0
" 26	911	"	"	"	25·0	31·0	19·0
" 27	911	"	"	"	24·9	32·0	15·0
" 27	911	"	"	"	25·9	32·0	18·0
" 27	911	"	"	"	27·0	32·0	17·0
" 30	911	"	"	"	24·2	31·0	18·0
" 30	911	"	"	"	25·4	30·0	18·0
" 30	911	"	"	"	26·0	31·0	18·0
" 31	911	"	"	"	25·9	32·0	19·0
" 31	911	"	"	"	25·2	31·0	16·0
" 31	911	"	"	"	26·9	32·0	16·0
Aug. 1	911	"	"	"	23·4	32·0	16·0
" 1	911	"	"	"	23·9	33·0	17·0
" 1	911	"	"	"	24·2	36·0	17·0
" 2	911	"	"	"	21·7	27·0	17·0
" 2	911	"	"	"	21·0	30·0	16·0
" 2	911	"	"	"	23·4	31·0	16·0
" 3	911	"	"	"	21·5	31·0	16·0
" 3	911	"	"	"	21·6	31·0	16·0
" 3	911	"	"	"	21·7	29·0	16·0
" 5	911	"	"	"	21·9	32·0	17·0
" 5	911	"	"	"	23·2	29·0	17·0
" 5	911	"	"	"	22·1	30·0	17·0
" 6	911	"	"	"	24·2	32·0	17·0
					23·9	36·0	15·0

CHART 6.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally Infected Dog, Strain II, Dog 690, taken on nine consecutive days from Rat 911



The curve of Strain II, Dog 690, is also eminently dimorphic, so much so that the presence of two species might be suspected, one with a maximum of 18 microns and the other with a maximum of 28 microns. If such were the case it could be argued that in Chart 3 of Strain I the long species had driven out the short But it will be shown later that this is probably not so that the difference is merely due to dimorphism and not to the mixture of two species of trypanosomes

Breadth—The following table gives the breadth of Strain II, Dog 690 —

Table XI.—Measurements of the Breadth of the Trypanosome of Naturally infected Dog, Strain II, Dog 690

Date	Experiment No	Animal	Number of trypanosomes measured	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1912	911	Rat	500	2 70	4 75	1 25

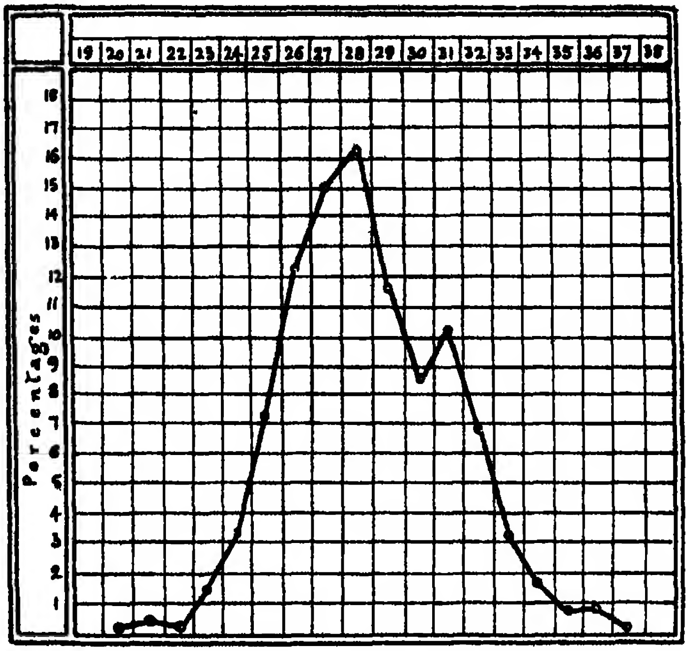
In regard to posterior-nuclear forms in this strain, there are practically none.

MORPHOLOGY OF THE NATURALLY INFECTED DOG STRAIN STRAIN III DOG 2033.

Table XII.—Measurements of the Length of the Trypanosome of Naturally Infected Dog, Strain III, Dog 2033.

Date	No of expt	Animal	Method of fixing	Method of staining	In microns. Average length.	Maximum length	Minimum length
1913							
April 10	2037	Rat	Osmic acid	Giemsa	29·6	34·0	24·0
" 10	2037	"	"	"	29·9	36·0	25·0
" 10	2037	"	"	"	30·2	36·0	24·0
" 11	2037	"	"	"	29·1	33·0	26·0
" 11	2037	"	"	"	29·5	38·0	25·0
" 11	2037	"	"	"	29·6	36·0	24·0
" 13	2037	"	"	"	29·7	35·0	25·0
" 13	2037	"	"	"	28·2	33·0	25·0
" 13	2037	"	"	"	29·6	38·0	25·0
" 14	2037	"	"	"	27·8	34·0	24·0
" 14	2037	"	"	"	29·7	37·0	25·0
" 14	2037	"	"	"	27·8	33·0	22·0
" 15	2037	"	"	"	28·1	32·0	25·0
" 15	2037	"	"	"	28·6	32·0	24·0
" 15	2037	"	"	"	27·5	31·0	25·0
" 16	2037	"	"	"	27·9	33·0	24·0
" 16	2037	"	"	"	28·1	33·0	24·0
" 16	2037	"	"	"	27·5	32·0	23·0
" 17	2037	"	"	"	26·8	31·0	21·0
" 17	2037	"	"	"	26·1	33·0	20·0
" 17	2037	"	"	"	26·1	29·0	23·0
" 19	2037	"	"	"	27·6	32·0	24·0
" 19	2037	"	"	"	28·5	36·0	25·0
" 19	2037	"	"	"	28·3	34·0	25·0
" 21	2037	"	"	"	27·9	31·0	22·0
					28·4	37·0	20·0

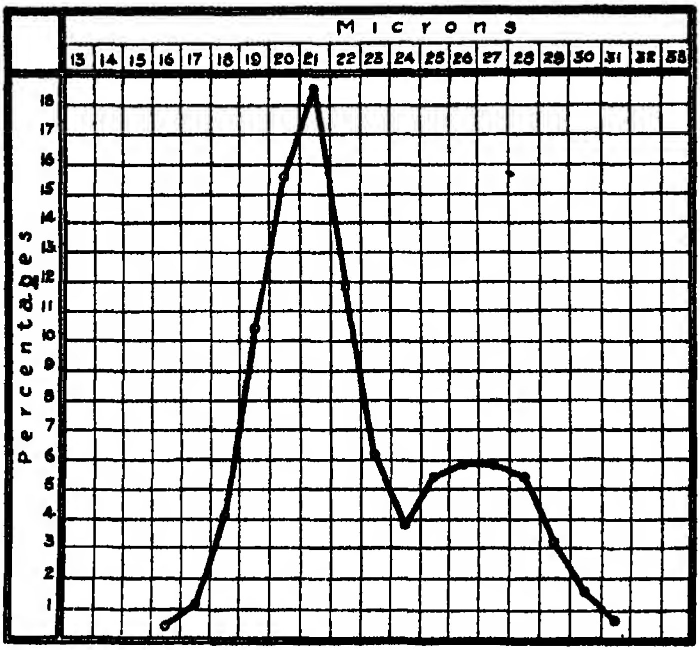
Chart 7.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally-Infected Dog, Strain III, Dog 2033, taken on nine consecutive days from Rat 2037.



This curve was taken from a rat which was inoculated directly from the naturally infected Dog 2033, and had therefore passed through no series of rats. Yet the curve is the same as that shown in Chart 4 after two years' passage through rats

It might be argued that this is really an infection with the larger of the two hypothetical species, the one having a maximum of 28 microns. It is to be regretted that this strain has died out, so that no further experimentation with it is possible It would have been interesting to pass it through other animals, in order to learn if any reversion to the short and stumpy form would take place. But if it is not possible to do this with Strain II, it is with Strain I, which was seen to change from a dimorphic type to a practically monomorphic type after two years' passage through rats When this almost monomorphic rat strain, as shown in Chart 4, is inoculated into a dog, a reversion to the original dimorphic type is brought about, as will be seen from the following chart:—

Chart 8.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Naturally Infected Dog, Strain I, Dog 48, taken from Dog 2498. This dog was inoculated from Rat 2471, which showed 95 per cent. long and slender forms.



In the blood of Rat 2471, Chart 4, the trypanosomes almost all belonged to the long and slender type. Now this is reversed, and the majority are short and stumpy. This goes against the theory that two species are being dealt with in this strain. Passage through the rat favours the production of a long and slender monomorphic type of trypanosome, whereas passage from the rat to the dog at once changes this to a dimorphic type, in which the short and stumpy form the greater number.

Breadth.—The following table gives the breadth of the trypanosome of Strain III, Dog 2033:—

Table XIII.—Measurements of the Breadth of the Trypanosome of Naturally Infected Dog, Strain III, Dog 2033.

Date.	Expt. No.	Animal.	Number of trypanosomes measured.	In microns.		
				Average breadth.	Maximum breadth.	Minimum breadth.
1913	2037	Rat	500	2·61	4·00	1·50

In Strain III there are no posterior-nucleated forms. This is not to be wondered at, as there are almost no short and stumpy forms, and it is only, or almost only, among them that posterior-nucleated trypanosomes are found.

Comparison of the Three Naturally Infected Dog Strains with One Another.

Table XIV.—Measurements of the Length of the Trypanosome of the Naturally Infected Dog, Strains I, II, and III.

Date	Expt No	Strain	Animal	Number of trypanosomes measured	In microns: Average length	Maximum length	Minimum length
1912	—	I	Rat	660	23 7	36 0	15 ·0
1912	911	II	„	500	23 ·9	36 0	15 0
1913	2037	III	„	500	28 4	37 ·0	20 0

Table XV.—Percentages of Short and Stumpy, Intermediate, and Long and Slender Forms in the Three Strains of the Naturally Infected Dog.

Date	Expt No	Strain	Animal	Number of trypanosomes measured	Short and stumpy, 15–21	Intermediate 22–24	Long and Slender, 25–37
					per cent	per cent	per cent
1912	—	I	Rat	660	47 1	10 2	42 7
1912	911	II	„	500	38 2	9 2	52 6
1913	2037	III	„	500	0 6	4 8	94 6

Strains I and II are similar, but Strain III differs so much from them that it would be useless to combine the three into one curve, the result would be misleading. In Strain III, as will be seen from Table XV, almost all are long forms.

Table XVI.—Measurements of the Breadth of the Trypanosome of the Naturally Infected Dog, Strains I, II, and III.

Date.	Expt No	Strain	Animal	Number of trypanosomes measured	In microns: Average breadth	Maximum breadth	Minimum breadth.
1912	1218	I	Rat	500	2 ·90	5 ·00	1 ·25
1912	911	II	„	500	2 79	4 75	1 ·25
1913	2037	III	„	500	2 61	4 ·00	1 ·50

Table XVII.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Naturally Infected Dog, Strains I, II, and III.

Date	Experiment No	Strain	Animal.	Percentage among short and stumpy forms.
1912	407	I	Rat	7·0
1912	911	II	"	0 1
1913	2087	III	"	0·0

COMPARISON OF THE NATURALLY INFECTED DOG STRAIN WITH THE HUMAN, WILD-GAME, AND WILD GLOSSINA MORSITANS STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND.

Table XVIII.—Measurements of the Length of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans* and Naturally Infected Dog Strains.

Strain	Number of trypanosomes measured	Animal	In microns		
			Average length	Maximum length	Minimum length
Human	5500	Rat	23·5	38·0	14 0
Wild-game	2500	"	22·6	35 0	15·0
Wild *G morsitans*	2500	"	22·6	35·0	15·0
Naturally infected dog	1660	"	25·5	37 0	15·0

Table XIX—Measurements of the Breadth of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans* and Naturally Infected Dog Strains.

Strain	Number of trypanosomes measured.	Animal.	In microns		
			Average breadth	Maximum breadth	Minimum breadth
Human	1500	Rat	2·6	5·00	1·25
Wild-game	1500	"	3 2	5·75	1·50
Wild *G morsitans*	1500	"	2·9	5·25	1·25
Naturally infected dog	1500	"	2·8	5·00	1·25

Table XX.—Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of the Human, Wild-game, Wild *Glossina morsitans*, and Naturally Infected Dog Strains

Date	Strain	Animal	Percentage among short and stumpy forms
1912	Human	Rat	17 8
1912	Wild game	"	26 2
1912	Wild *G. morsitans*	"	12 5
1912	Naturally infected dog	"	2 4

COMPARISON OF THE MORPHOLOGY OF THE NATURALLY INFECTED DOG STRAIN WITH THE OTHER STRAINS OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND

At the outset it may be stated that it is impossible to separate the Naturally Infected Dog strain from the other strain by microscopical examination As far as can be made out it is identical in shape, size and position of nucleus and micronucleus, contents of cell, and disposal of the undulating membrane

Three plates are given at the end of this paper to illustrate the morphology of this strain, and if they are compared with the plates given of the other strains* this statement will be borne out

On the other hand, there are very few posterior-nuclear forms, although in one instance they ran up to 23 per cent., and, as a rule, the thick, blunt-ended type is not so common in this strain as in the others But for all practical purposes it must be concluded that the Naturally Infected Dog strain is so similar in appearance to the others that it would be impossible to separate it by morphology alone

How this aberrant strain arose in these three chronically infected dogs it is impossible to say. If it had been found anywhere else—in man, game, or fly—the position would have been simplified But in none of them did anything like the Naturally Infected Dog strain appear. It was thought that perhaps the long sojourn in the blood of the dog had modified and weakened this strain, and attempts were made to prove this, but without success. All the dogs inoculated with the ordinary strains died in a few weeks, and inoculations from those which lingered longest showed no signs of weakening or change of any kind.

* 'Roy Soc. Proc,' B, vol. 87, p 35 (1913). *Ibid*, B, vol. 87, p. 493 (1914) "Description of a Strain of *Trypanosoma brucei* from Zululand."

CONCLUSIONS.

1. The Naturally Infected Dog strain differs slightly from the other strains of the trypanosome causing disease in man in Nyasaland, in that there are fewer of the posterior-nucleated, blunt-ended forms which are sometimes so much in evidence in the ordinary strains.

2 Taking into consideration the fact that this strain was only found in three chronically infected dogs, it is concluded that it is an aberrant strain of the widely spread species *T brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland

DESCRIPTION OF PLATES

Trypanosome of Naturally Infected Dog
Plate 9 —Short and Stumpy, Non-flagellated Forms
Plate 10.—Intermediate Forms.
Plate 11 —Long and Slender Forms
× 2000

The Trypanosome causing Disease in Man in Nyasaland. The Naturally Infected Dog Strain. Part II.—*Susceptibility of Animals.*

By Surgeon-General Sir DAVID BRUCE, C.B., F R S, A M.S, Major A. E. HAMERTON, D S.O, and Captain D P WATSON, R.A M C., and Lady BRUCE, R.R C (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

(Received April 16,—Read June 25, 1914)

INTRODUCTION.

In a previous paper* the morphology of the three strains of this trypanosome, from three naturally infected dogs, was described, and the strains compared with each other and with the Human strain

This paper describes the action on various animals of the three strains and tabulates a comparison with the Human strain

The first strain—Dog 48—was studied in a fairly large number of animals, but the second and third in few, as both were accidentally lost.

* 'Roy. Soc. Proc.,' B, vol. 88, p. 111 (1914).

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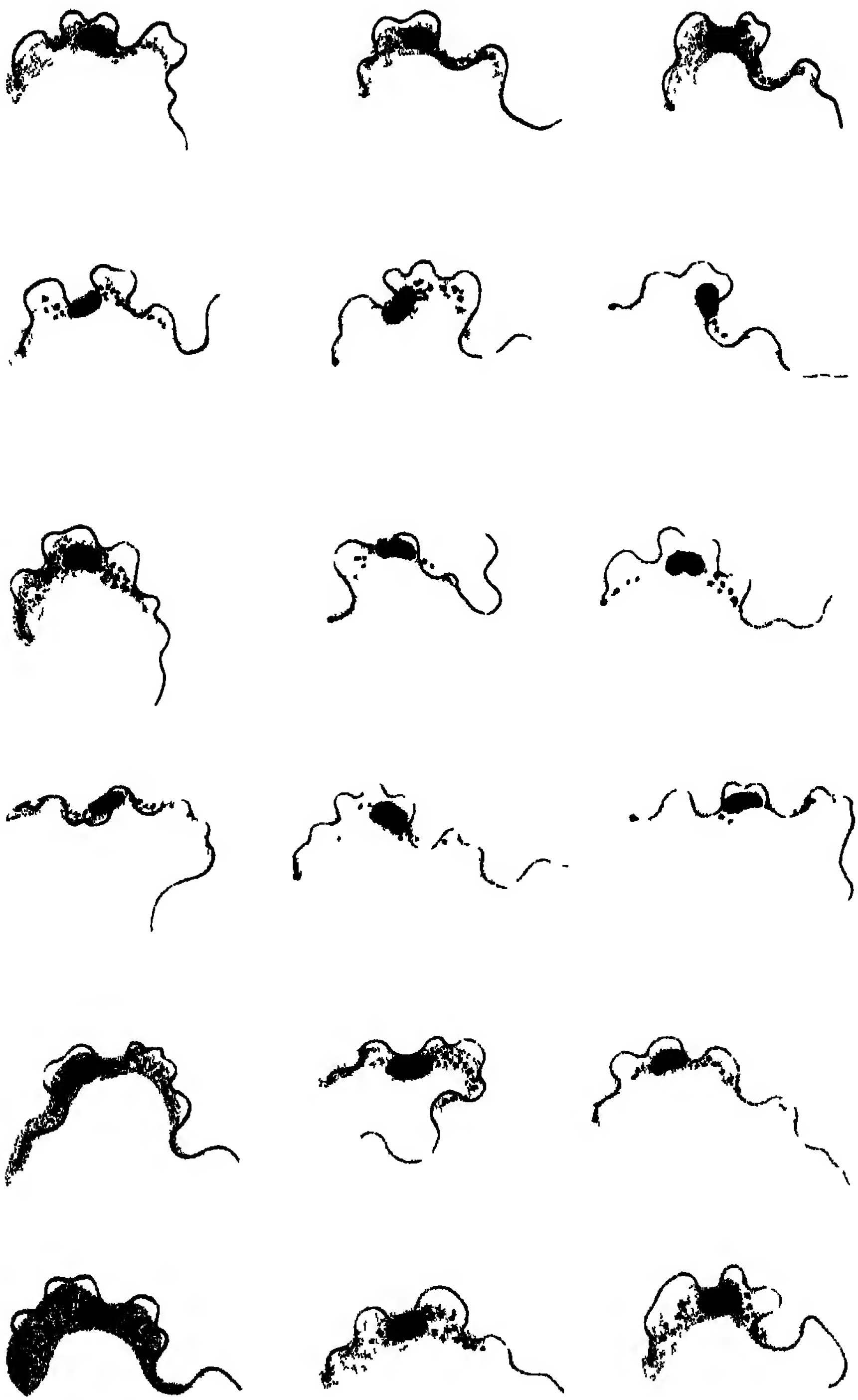
SHORT AND STUMPY FORMS



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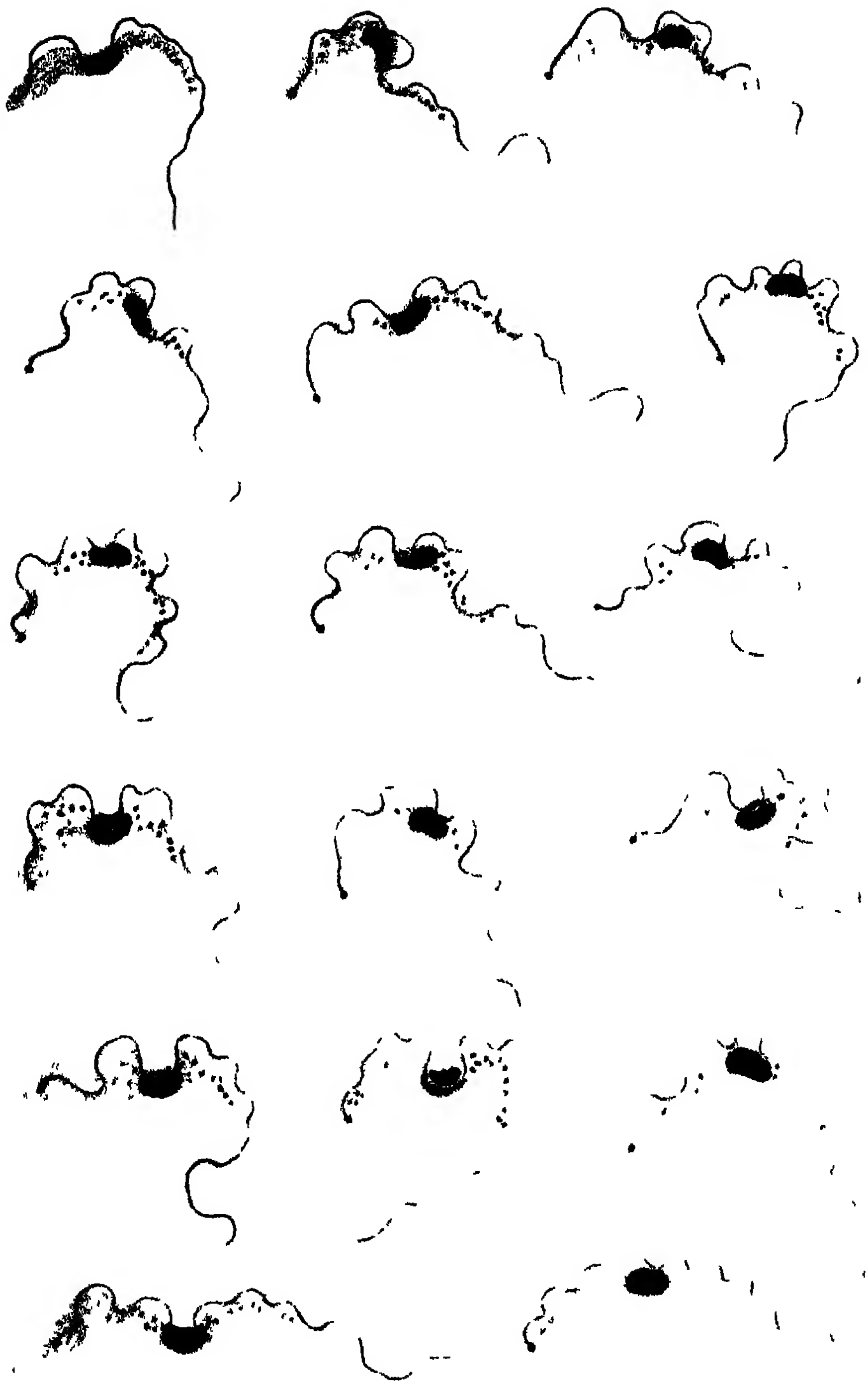
INTERMEDIATE



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LONG AND SLENDER FORMS

Susceptibility of Animals to the Naturally Infected Dog Strain.

I. *Strain I, Dog* 48

Table I

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Cattle		
1912					
Mar 6	314	From Dog 210	—	—	Never showed trypanosomes
" 6	315	" 210	—	—	" " "
April 13	314	From Rat 311	12	—	Still alive after 335 days
" 13	315	" 311	—	—	Never showed trypanosomes
			Goat		
Mar 6	275	From Dog 210	—	—	Never showed trypanosomes
" 6	277	" 210	—	—	" " "
April 5	275	From Rat 312	24	—	Accidentally killed
" 5	277	" 312	—	—	Never showed trypanosomes
" 20	427	" 392	10	—	Still alive after 277 days
" 20	432	" 392	26	—	" " "
1913					
Mar 21	2008	" 1991	—	—	Never showed trypanosomes
" 21	2009	" 1991	27	—	Still alive after 251 days
" 21	2010	" 1991	—	—	Never showed trypanosomes
" 21	2011	" 1991	17	—	Died of pneumonia
" 21	2012	" 1991	—	—	Never showed trypanosomes
" 21	2013	" 1991	—	—	" " "
" 21	2014	" 1991	24	—	Still alive after 251 days
" 21	2015	" 1991	17	—	" " "
" 21	2016	" 1991	—	—	Never showed trypanosomes
" 21	2017	" 1991	—	—	" " "
			Sheep		
1912.					
April 20	456	From Rat 392	5	—	Still alive after 340 days
" 20	457	" 392	10	64	Died of Strain I
			Antelope		
1913					
May 21	2059	From Rat 2024	13	—	Still alive after 250 days
			Monkey		
1912					
Mar. 6.	318	From Dog 210	—	—	Never showed trypanosomes
April 5.	318	From Rat 312	—	—	" " "
" 20.	50	From Dog 317	—	—	" " "
" 20	453	From Rat 392	—	—	" " "
Oct. 29	1583	" 1401	—	—	" " "
" 29	1584	" 1401	6	—	Still alive after 148 days
Nov. 22	1629	From Monkey 1584	—	—	Never showed trypanosomes.
" 27	1630	" 1584	10	—	Still alive after 124 days.

* Duration includes the days of incubation; it dates from the day of inoculation

Table I—*continued.*

Date	No of expt.	Source of virus	Period of incubation, in days.	Duration of disease, in days *	Remarks.
		Monkey—*continued.*			
1913					
Jan. 22	1792	From Rat 1741	5	—	Still alive after 186 days.
" 22	1793	" 1741	5	—	" " "
" 22	1794	" 1741	—	—	Never showed trypanosomes.
" 22	1798	From Monkey 1630	—	—	" " "
Feb 28	1794	From Rat 1945	—	—	" " "
" 28	1798	" 1945	—	—	" " "
May 22	1794	From Monkey 2131	—	—	" " "
" 22	1798	" 2131	—	—	" " "
June 11	1794	" 2184	—	—	" " "
" 11	1798	" 2184	—	—	" " "
		Dog			
1912					
Feb 17	210	From Rat 67	8	25	Died of Strain I
Mar. 6	317	From Dog 210	—	—	Never showed trypanosomes.
" 13	331	From Rat 67	—	—	" " "
April 6	317	" 312	12	16	Died of Strain I
" 13	331	" 311	12	46	" "
" 20	458	From Dog 317	—	—	Never showed trypanosomes
" 20	459	" 317	19	—	Still alive after 224 days
Sept 6	1253	From Rat 1218	6	—	" " 120 "
Oct 29	1525	" 1491	18	30	Died of Strain I
" 29	1526	" 1491	23	58	" "
" 29	1527	" 1491	—	—	Never showed trypanosomes.
" 29	1528	" 1491	13	21	Died of Strain I
" 29	1529	" 1491	13	47	" "
" 29	1530	" 1491	16	—	Still alive after 148 days.
1913					
Jan 22	1795	From Rat 1741	5	29	Died of Strain I.
" 22	1796	" 1741	8	35	" "
" 22	1797	" 1741	22	—	Still alive after 131 days.
April 14	2054	Laboratory-bred flies	7	30	Died of Strain I
May 29	2107	From Dog 2054	11	16	" "
" 29	2198	" 2054	4	18	" "
Dec 30	2483	From Rat 2471	14	34	" "
1914					
Jan 26	2498	From Dog 2483	4	11	Died of Strain I.
		Average	11·6	29 0	
		Rabbit.			
1912					
Mar 30	389	From Rat 67	9	256	Died of Strain I
" 30	390	" 67	17	109	" "
		Average	13·0	182·5	
		Guinea-pig			
Mar. 6	313	From Dog 210	—	—	Never showed trypanosomes
April 5	313	From Rat 312	—	—	" "
" 20	460	" 392	—	—	" "
" 20	461	" 392	—	—	" "
Oct. 29	1531	" 1491	—	—	" "
" 29	1532	" 1491	—	—	" "
Nov 13	1581	" 1492	—	—	" "
" 13	1582	" 1492	—	—	" "

* Duration includes the days of incubation, it dates from day of inoculation.

Table I—*continued.*

Date	No of expt.	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
		Guinea-pig—*continued*			
1913					
Jan. 17	1775	„ 1734	—	—	Never showed trypanosomes
June 17	2228	„ 2215	—	—	„ „
„ 17	2229	„ 2215	6	—	Still alive after 246 days
July 22	2307	„ 2285	—	-	Never showed trypanosomes
„ 22	2308	„ 2285	—	—	„ „
„ 31	2307	„ 2285	—	—	„ „
„ 31	2308	„ 2285	—	—	„ „
		Rat			
1912					
Mar 26	311	From Rat 67	13	18	Died of Strain I
„ 26	312	„ 67	9	60	„ „
„ 30	391	„ 67	9	21	„ „
„ 30	392	„ 67	9	21	„ „
April 5	407	„ 312	10	54	„ „
„ 20	462	„ 407	10	25	„ „
May 25	585	„ 407	5	28	„ „
June 4	670	„ 585	6	27	„ „
July 2	786	„ 670	6	45	„ „
Aug 2	1020	„ 786	6	30	„ „
„ 30	1218	„ 1020	3	94	„ „
Oct 19	1492	„ 1218	9	54	„ „
Dec 12	1687	„ 1492	4	16	„ „
„ 28	1719	„ 1687	9	32	„ „
1913					
Jan 3	1734	„ 1570	9	36	„ „
„ 3	1735	„ 1570	6	39	„ „
„ 7	1741	„ 1719	4	15	„ „
„ 9	1749	„ 1719	4	18	„ „
„ 27	1814	„ 1749	7	16	„ „
Feb 10	1855	„ 1814	3	20	„ „
„ 22	1945	„ 1855	5	17	„ „
Mar 7	1985	„ 1945	6	20	„ „
„ 25	2022	„ 1985	6	16	„ „
„ 25	2023	„ 1985	6	18	„ „
„ 25	2024	„ 1985	6	19	„ „
April 10	2070	„ 2023	4	12	„ „
„ 22	2105	„ 2070	6	20	„ „
May 7	2124	„ 2105	3	8	„ „
„ 13	2134	From Dog 2054	2	12	„ „
„ 25	2188	From Rat 2133	3	7	„ „
„ 15	2168	„ 2124	7	17	„ „
„ 18	2138	From Dog 2054	6	10	„ „
June 10	2214	„ 2197	3	10	„ „
„ 18	2230	From Rat 2214	8	10	„ „
Aug. 18	2389	„ 2280	8	48	„ „
Sept. 6	2409	„ 2389	5	49	„ „
„ 16	2413	„ 2409	6	70	„ „
Oct. 28	2425	„ 2413	5	64	„ „
Nov 12	2432	„ 2425	6	38	„ „
Dec 20	2471	„ 2432	6	22	„ „
1914					
Jan. 16	2484	„ 2471	7	16	„ „
		Average	6 2	28·6	

* Duration includes the days of incubation, it dates from the day of inoculation

II *Strain II, Dog* 690.

Table II

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Monkey		
1913 Sept 13	1814	From Dog 690	—	—	Never showed trypanosomes.
„ 13	1815	„ 690	—	—	„ „
			Dog		
1912 June 17	690	Naturally infected	?	—	Recovered
			Guinea-pig		
1913 Sept 13	1316	From Dog 690	—	—	Never showed trypanosomes
„ 13	1317	„ 690	—	—	„ „
			Rat		
1912 July 18	911	From Dog 690	7	30	Died of Strain II.

* Duration includes the days of incubation, it dates from the day of inoculation

III *Strain III, Dog* 2033

Table III

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Goat		
1913 May 21	2174	From Rat 2089	12	71	Cause of death uncertain.
„ 21	2175	„ 2089	—	—	Never showed trypanosomes
„ 21	2176	„ 2089	—	—	„ „
„ 21	2177	„ 2089	—	—	„ „
„ 21	2178	„ 2089	12	—	Died of pneumonia
			Monkey		
May 14	2161	From Rat 2091	—	—	Never showed trypanosomes
„ 14	2162	„ 2091	8	—	Recovered
„ 14	2163	„ 2091	—	—	Never showed trypanosomes
„ 14	2164	„ 2091	—	—	„ „
„ 14	2165	„ 2091	12	—	Recovered.
June 14	2161	From Dog 2157	—	—	Never showed trypanosomes.
„ 14	2164	„ 2157	—	—	„ „

* Duration includes the days of incubation; it dates from day of inoculation.

Table III—*continued.*

Date.	No of expt.	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Dog		
Mar 28	2033	Naturally infected	?	?	Died April 1
May 14	2156	From Rat 2091	15	40	Died of Strain III.
" 14	2157	" 2091	8	33	" "
" 14	2158	" 2091	8	26	" "
" 14	2159	" 2091	15	93	" "
" 14	2160	" 2091	15	102	" "
		Average	12 2	58 8	
			Guinea-pig.		
Mar. 28	2039	From Dog 2033	—	—	Never showed trypanosomes
" 28	2040	" 2033	—	—	" "
May 21	2180	From Rat 2089	22	—	Recovered
" 21	2181	" 2089	—	—	Never showed trypanosomes
			Rat.		
Mar 28	2037	From Dog 2033	13	73	Died of Strain III
Apr 16	2089	From Rat 2037	5	15	" "
" 16	2090	" 2037	5	87	" "
" 16	2091	" 2037	5	28	" "
May 14	2167	" 2091	8	57	" "
Oct. 23	2426	From Guinea pig 2180	11	19	" "
		Average	7 8	46 5	

* Duration includes the days of incubation, it dates from the day of inoculation

Disease set up in various Animals by the Trypanosome causing Disease in Man in Nyasaland. The Naturally Infected Dog Strain

Ox.—This trypanosome does not appear to be virulent to the ox Four experiments were made. The trypanosomes appeared in the blood of one of the oxen, and it was returned as "Recovered" after being under observation for 335 days. The parasites were only seen on three occasions in this ox, and then only in scanty numbers

Goat—The trypanosome also has little effect on goats Twenty-one were inoculated Of these 12 proved refractory, five showed the trypanosomes in their blood on one or two occasions in very scanty numbers, and were returned as "Recovered" after being under observation for nearly a year, four died, one from the result of an accident, two from pneumonia, and the remaining one only once showed the trypanosomes, and as no *post-mortem* examination was made it is impossible to say what was the cause of death. It may therefore be said that not a single goat of the 21 died of the disease.

Sheep.—Two sheep were inoculated. One recovered; the other died after 64 days, probably of the disease.

Monkey.—This trypanosome has little or no effect on monkeys. Twenty-seven were used as experimental animals. Twenty-one proved refractory, the remaining six were returned as "Recovered" after being under observation for several months.

Dog.—This strain has become, after several passages, virulent to dogs. Twenty-eight were used for experiment. Nineteen died, on an average, in 36.8 days (11 to 102), four never showed trypanosomes in their blood; and five recovered. The *post-mortem* appearances are the same as those found in Nagana: enlargement of the spleen, gelatinous œdema about the vessels at the base of heart, petechiæ of mucous membranes, and corneal opacity.

Rabbit.—Only two were inoculated. Both died, one after 109 days, the other after 256 days. Both showed corneal opacity and presented the same symptoms as those described in Nagana rabbits, but in a much milder degree.

Guinea-pigs.—The guinea-pig, like the monkey, is almost refractory to this strain. Twenty-one animals were inoculated. Nineteen of these proved refractory, and the remaining two only showed trypanosomes on one occasion and appear to have recovered. Rats inoculated with their blood remain unaffected.

White Rat.—This strain is virulent to rats. Forty-eight were inoculated, and all died, on an average, in 30.8 days (7 to 94), with enormous enlargement of spleen, and the blood swarming with trypanosomes.

Comparison of the three Strains of the Trypanosome of the Naturally Infected Dog Strain in Regard to their Virulence towards various Animals.

Table IV.—The Average Duration, in Days, of the Disease in various Animals of the three Strains. The letter R means that the animal is refractory.

Strain	Ox	Goat.	Sheep	Monkey	Dog.	Rabbit	Guinea-pig	White rat
I	R	R.	64	R.	29	182	R	29
II	—	—	—	R.	—	—	R.	30
III	—	R	—	R	59	—	R	46

Table V.—The Average Duration, in Days, of the Disease in various Animals of the three Strains combined. The letter R stands for "refractory."

	Ox	Goat	Sheep	Monkey	Dog	Rabbit	Guinea pig	White rat
Average duration, in days	R	R	64	R	37	182	R	31
Number of animals employed	4	21	2	27	19	2	21	48

Table VI.—The Percentages of Recoveries in various Animals infected with the Naturally Infected Dog Strain. Three strains combined.

	Ox	Goat	Sheep	Monkey	Dog	Rabbit	Guinea-pig	White rat
Percentages	100	100	50	100	21	0	100	0
Number of animals employed	1	7	2	6	24	2	2	48

From Table VI it will be seen that the Naturally Infected Dog strain is not fatal to oxen, goats, monkeys, or guinea-pigs, whereas it killed 79 per cent. of the dogs and 100 per cent. of the white rats.

Comparison of the Trypanosome of the Naturally Infected Dog Strain with the Trypanosome causing Disease in Man in Nyasaland (Trypanosoma brucei vel rhodesiense).

Table VII.—The Average Duration of Life, in Days, of various Animals infected with the Naturally Infected Dog Strain and the Human Strain. The letter R stands for "refractory."

Strain	Ox	Goat	Monkey	Dog	Rabbit	Guinea pig	White rat
Naturally infected dog	R.	R	R.	37	182	R	31
Human ..	134	42	26	34	28	67	30

It is curious that this strain, although evidently harmless to oxen, goats, monkeys, and guinea-pigs, is quite as virulent as the Human strain to dogs and rats.

Table VIII.—The Percentages of Recoveries in various Animals infected with the Naturally Infected Dog Strain and the Human Strain.

Strain	Ox	Goat	Monkey	Dog	Rabbit.	Guinea-pig	White rat.
Naturally infected dog	100	100	100	21	0	100	0
Human	80	0	0	0	0	0	0

This shows the great difference in regard to action on animals which exists between the Naturally Infected Dog strain and the Human strain,* and if similar tables referring to other strains—for example, the Zululand 1913 Strain†—be compared, the same difference is found. It might be said that this alone is sufficient to make it rank as another species, and, as already mentioned, if this strain had been found among the wild game and wild *Glossina morsitans* in Nyasaland, this would have been justified. It was, however, only found in three chronically infected dogs, and so it is thought best with our present knowledge to include it among the strains of *Trypanosoma brucei vel rhodesiense*.

If in the future it should be decided to give it specific rank the name *T. anceps* is suggested. This name seems appropriate on account of the uncertainty which exists as to the classification of this trypanosome.

CONCLUSIONS.

1. The Naturally Infected Dog strain is fatal to dogs, rabbits, and white rats, but oxen, goats, monkeys, and guinea-pigs appear to be refractory.

2. The Commission is of opinion that this is an aberrant or exceptional variety or strain of the trypanosome causing disease in man in Nyasaland—*T. brucei vel rhodesiense*.

* 'Roy. Soc. Proc.,' B, vol. 87, p. 35 (1913).

† *Ibid.*, B, vol. 87, p. 493 (1914).

The Vapour-Pressure Hypothesis of Contraction of Striated Muscle.

By H. E Roaf.

(Communicated by Prof C S Sherrington, F R S Received April 16,—Read June 18, 1914.)

(From the Laboratory of Physiology, St Mary's Hospital Medical School)

In 1854 Graham suggested that muscular contraction might be due to osmotic influences (6) In 1878 FitzGerald suggested, on the other hand, that muscular contraction might be due to changes in tension at the surface of the fibrils of muscle (4) The application of these two hypotheses to the problem of muscular contraction is still under investigation and discussion

Two principal objections have been raised to the osmotic explanation —(1) "Neither theoretically nor practically is it possible to construct a model in the manner imagined by McDougall, which will, on being distended, produce anything near the shortening which is observed in living muscle Living muscle may contract certainly to one-third of its length" (25) (2) 'It is impossible to conceive that water will flow into the sarcostyles from the sarcoplasm, not in a third or a tenth of a second, only, but as in the case of the wing muscles of insects, in less than one-two thousandth of a second" (10) These two objections seem to be shared by Bernstein (2), Macallum (9), and Schafer (24).

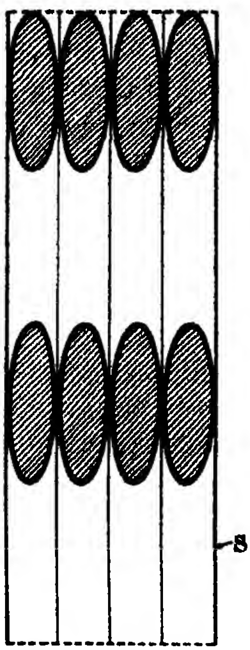
On the other hand, Macdonald (11), Macdougall (13), Pauli (15), and Zuntz (27), support the hypothesis that muscular contraction is due to a rise of osmotic pressure

The structure of striated mammalian muscle is generally agreed to be a series of fibrils suspended in sarcoplasm, the whole surrounded by the sarcolemma The fibrils consist of alternating bands of anisotropous and isotropous substance, the former corresponding to the portion of the fibril which is contained in the dim band, the latter to the light band of the fibril (26) Accepting this, the model that I wish to describe assumes that during contraction lactic acid is formed, causing the portion of the fibril contained in the dim band to swell and become spherical (see figure) Such a process is exemplified by a parchment paper osmometer containing a protein solution If the osmometer is placed in a dilute solution of acid, the acid diffuses in and causes an increased absorption of water, with a rise of pressure

One explanation of this rise of pressure is that, as the acid diffuses into the

osmometer, it unites with the protein to form an ionising salt. Of this salt the protein ion cannot pass through the parchment paper membrane, and the acidic ion is held back by the opposite electrical charge on the protein. The pressure inside the osmometer consists of the sum of the protein and acidic ions and the free acid, the pressure outside the osmometer consists of the free acid alone. Therefore the excess pressure inside is due to the protein and acidic ions (14, 17, 18, 20).

If in calculating the rate of contraction we assume that the lactic acid is liberated in the isotropous substance, and that it must diffuse into the anisotropous substance, we are using the most unfavourable conditions for the



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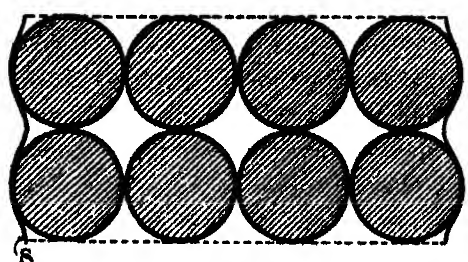

B

Diagram illustrating to scale the osmotic contraction of two segments of four fibrils of frog's sartorius muscle. Dim band 0·9 μ long, light band 0·9 μ long and fibrils 0·3 μ diameter. A, relaxed, B, contracted condition. S, sarcolemma, shaded portion anisotropic substance, unshaded portion isotropic substance.

osmotic hypothesis. The anisotropous substance is considered to be an ellipsoid, but calculations assuming that the anisotropous substance is cylindrical give practically the same results.

In calculating the extent and rate of contraction the results will depend upon the dimensions of the structures concerned. Measurements made by Dr F. O'B Ellison on frog's sartorius show that there the length of the dim band is 0·9 μ, the length of the light band is 0·9 μ, and the diameter of the fibril is approximately 0·3 μ. As the diameter of the fibril and relative lengths of the dim and light bands may vary in different muscles, the calculations are made so as to show what variations these differences may allow in the extent and rate of contraction. In the Table (p. 146), therefore,

the calculations are made for ellipsoids 0 9 μ long and 0 4, 0 3, 0·2, and 0·1 μ in diameter, that is with half-axes, a and b, 0 45 μ and 0 20, 0·15, 0 10, and 0 05 μ respectively

We can assume that the ellipsoid swells to become a sphere of the same surface area, that the ellipsoid is really an extended sphere with the walls thrown into folds, or that it has inextensible longitudinal fibres and extensible circular fibres The first assumption is not valid, as one cannot imagine a surface area of constant magnitude which would be so mobile as to change from the surface of an ellipsoid to that of a sphere The other two assumptions give the same results and they form the mechanical basis of the ensuing calculations

We can simplify the description by dealing with one dim band and an adjacent light band, since the result is the same even if one dim band is associated with the two adjacent half light bands

Extent of Contraction

The quotation given above refers specifically to Macdougall's hypothesis (13), but the same criticism seems to be tacitly applied to all osmotic hypotheses

Assuming that the ellipsoid becomes a sphere, the longitudinal perimeter of the ellipsoid (Table, p 146, column 3) will be the circumference of the sphere The dim band, therefore, shortens in the ratio of the length of ellipsoid to diameter of sphere, that is as $2a$ is to $2r$

Not only does the dim band shorten but the area of the dim band increases The fibrils being closely packed together, the increase in area of dim band will be proportional to the squares of the radii of the ellipsoid and sphere respectively, or as b^2 is to r^2 The relative volumes of the dim band in the two conditions are given by this ratio multiplied by the corresponding length of the band These relative volumes, $2a \times b^2$, and $2r \times r^2$, are given in the Table (columns 4 and 8)

The volume of the dim band is more than doubled as the result of contraction (column 9) The extent of contraction can be estimated by assuming—(a) that, as measured above, the light band is exactly the same length as the dim band, or (b) that the length of the light band is such that, during contraction, the whole of the muscle can be just absorbed by the dim band. These figures are given in the Table (columns 10, 11, and 12). The length of the muscle fibre which can be absorbed by the dim band is easy to calculate, as the area of the light band is exactly the same as that of the dim band before contraction, hence the relative length is the number of times that the volume of the contracted dim band contains the volume of the dim band before contraction

M 2

A model to illustrate the above principles was made (22) by enclosing four rubber balloons in silk bags and suspending them from a disc; a rubber cylinder was placed round these, extending from the supporting disc to a similar disc below The whole was filled with water: a certain amount of water was removed from the cylinder surrounding the balloons and the same volume was injected into the balloons The total volume was thus unchanged and the model contracted to 50 per cent of its original length By more careful construction there is no doubt that a greater degree of contraction can be produced

The diagram shows, to scale, the contraction when ellipsoids 0·9 μ long and 0·3 μ in diameter become spheres of the same circumference, the light bands before contraction being of the same length as the dim band

The results of the calculations in this section are independent of the actual dimensions, but they depend on the relative dimensions The results in the following section, however, depend on the absolute values

Rate of Contraction

Graham pointed out that "in minute microscopic cells the osmotic movements should attain the highest velocity, being mainly dependent upon the extent of the surface" (7) This statement can be amplified by calculating the rate at which the ellipsoids would swell to form spheres.

The amount of liquid that is absorbed (column 13) is the difference between the volume of the sphere (column 6) and the volume of the ellipsoid (column 2) This amount of liquid passes through a surface which can be taken to be of the same extent as the surface of the sphere (column 7)

To calculate the rate at which absorption of water may take place through the membrane we may utilise the data which I obtained in some direct determinations of the osmotic pressure of hæmoglobin solutions (17, 20). The surface area of the parchment paper was 19 sq cm and osmotic equilibrium was attained in about three days by the absorption of about 5 c.c of water

If the rate of diffusion into the anisotropic substance is the same as the rate of diffusion through the parchment paper, the length of time until equilibrium will be reached will be directly proportional to the volume of liquid passing in, and inversely proportional to the surface area through which the liquid can pass The increase in volume when the ellipsoids become spheres (column 13) and the surface of the spheres (column 7) are given in the Table

In order to compare the increase in volume and surface of the osmometer with the corresponding measurements of the ellipsoids, the measurements in

centimetres must be converted into microns that is, 5 must be multiplied by $(10000)^3$ and 19 by $(10000)^2$.

The ratio,

$$\frac{5\times(10000)^3}{19\times(10000)^2} + \frac{\text{Increase in volume of ellipsoid}}{\text{Surface of sphere}},$$

denoting the relative surface through which unit volume passes into the anisotropic substance compared with unit volume through unit surface in the osmometer, is given in column 14 of the Table

In calculating the rate of diffusion we must distinguish between two processes, namely, the rate at which lactic acid diffuses from the isotropous into the anisotropous substance, and the rate at which water diffuses from the isotropous into the anisotropous substance

In each case the rate of diffusion must depend on the driving force, that is, the difference of pressure divided by the distance Let us assume that in the osmometer there is perfect mixing, so that the distance is the thickness of the parchment paper, 43 thicknesses of dry parchment paper were measured in a screw micrometer, their thickness was 3 7 mm, each piece is, therefore, at least 0 086 mm thick. As the parchment paper swells when wet, the actual thickness must be greater than this

Further, let us assume that the anisotropous substance is a gel, and let us take the greatest distance, namely, the radius of the sphere (column 5), as the distance that determines the driving force in the ellipsoid If the difference of pressure between the anisotropous substance and the isotropous substance were the same as that between the contents of an osmometer and its surrounding fluid, the driving force (being inversely as the distances across which the force acts) would be, as shown in the Table (column 15), very much greater in the case of the muscle fibre, in other words, the osmotic gradient would be much steeper

If acid is placed with the hæmoglobin inside the osmometer, equilibrium is reached in two to three days (17, 20), but when the acid is placed outside, the pressure reaches its maximum more gradually, depending on the rate at which acid enters the osmometer Let us assume that the latter arrangement corresponds with the conditions existing inside a muscle fibre, let us take three days as the period required to reach osmotic equilibrium once the pressure has been produced, and let us take 14 days as the period required for the acid to diffuse into the osmometer and cause a rise of pressure.

As the parchment paper is saturated with water the rate at which water enters the outer surface of the parchment paper determines the rate at which water enters the osmometer, that is, the rate of entry is proportional

to the osmotic gradient The time required to reach osmotic equilibrium in the fibril (column 17) is calculated by dividing three days, in seconds, by the relative surface through which unit volume passes (column 14) and also by the relative driving force (column 15)

In the case of the diffusion of acid, the acid must diffuse through the thickness of the paper before it can affect the contents of the osmometer Therefore, in calculating the time required to reach equilibrium, the distance must be taken into account a second time For this reason the equilibrium for acid will be reached in inverse proportion to the square of the distance (square of column 15) The length of time required to reach equilibrium for lactic acid in the muscle (column 16) is found by dividing 14 days, in seconds, by the relative surface through which unit volume passes (column 14), and also by the squares of the figures in column 15

In order to control the above calculation the rate of diffusion of lactic acid was measured by allowing it to diffuse into gelatine containing an indicator A 2-per-cent solution of gelatine was coloured by Congo red, and tubes 3 mm in diameter were filled with it After the gelatine had solidified, the tubes were cut into short lengths and placed into 25 c c. of solution The length of tube in which the indicator had changed colour was measured at definite time-intervals

Two tubes were placed in each flask, two flasks of each solution were used, and there were two ends to each tube, therefore the measurements are the average of eight determinations in each case

	0 05 N lactic acid	0·02 N lactic acid	0·01 N lactic acid.
hours	mm.	mm	mm.
1	3 4	2 6	2 0
4	7·0 (2 × 3 5)	5 5 (2 × 2·75)	4·0 (2 × 2·0)
25	17·0 (5 × 3·4)	14·0 (5 × 2·8)	10 1 (5 × 2·0)

A second experiment was carried out, but neutral red with sufficient alkali to cause a yellow colour was used. The diffusion was slightly slower, possibly because some of the acid was lost by neutralising the alkali

	0·05 N lactic acid	0·02 N lactic acid	0·01 N lactic acid
hours	mm	mm	mm
1	3 2	2 3	1·6
4	6 2 (2 × 3 1)	4 6 (2 × 2 3)	3 4 (2 × 1 7)
25	15 4 (5 × 3·1)	12 1 (5 × 2 4)	9·9 (5 × 2·0)

The results show very clearly that the time required for diffusion is

proportional to the square of the distance, therefore we can calculate the rate of diffusion of lactic acid in muscle fibre

Taking the rate of diffusion as between 2 and 3 mm. for the first hour, we can calculate the time for the acid to diffuse one micron as follows:—A micron is 0·001 mm., therefore the acid would diffuse one micron in between $(1/2000)^2$ and $(1/3000)^2$ of an hour, or between 0·0009 and 0·0004 of a second respectively. The radius of the spheres is about one-third of a micron, therefore the time for the acid to diffuse into the anisotropic substance is one-ninth of the above, or 0·0001 and 0·00004 of a second respectively.

These times are somewhat less than those given in the Table (column 16). In the Table the calculation is for the establishment of equilibrium, but these figures show the time for diffusion of just sufficient acid to cause a change of colour of the indicator. Considering this difference and the different ways in which the times were calculated the agreement is very satisfactory.

If we sum the two time-intervals (columns 16 and 17) we get the total time for complete osmotic contraction of muscle, if the two processes are successive (column 18). As the processes would be more or less concurrent, the time would actually be less.

In spite of the fact that in these calculations, wherever there was any doubt, the assumptions were made to the disadvantage of the view now defended, it will be seen that if the maximum contraction of frog's sartorius requires at least 0·04 second there is more than sufficient time for the contraction to be the result of osmotic swelling of the anisotropic substance.

The calculation for the wing muscles of insects has not been attempted owing to the lack of data. Although the fibrils are coarser the presence of minute canals in the anisotropous substance must be remembered, so that probably the results would be equally convincing. If it were permissible to consider the rate of diffusion of water as proportional to the square of the distance the ellipsoids would become spheres with sufficient rapidity to allow ample time for the contraction of insect's muscle.

Absolute Force of Muscle.

In the preceding discussion the term osmotic pressure has been used, but any other process which produces a lowering of vapour pressure could be used as the force by which water is moved into the anisotropous substance. In order, however, to make the discussion more concrete, I may quote the following experiment as showing the effect of lactic acid on the osmotic pressure of a protein (14, 18). As the proteins of muscle are difficult to obtain in an unaltered condition I have used hæmoglobin. It is more sensitive to reagents than the proteins of serum (21).

Table illustrating the Osmotic Possibilities of Muscle

All measurements of length, surface, and volume are given in μ, μ^2, and μ^3 respectively ($\mu = 0·001$ mm). In calculating the extent of contraction the relative dimensions are all that are required, but in calculating the rate of contraction the absolute measurements are required

	1. Ellipsoid: Major axis a	1. Ellipsoid: Minor axis b	2. Volume of ellipsoid. $\frac{4}{3}\pi a b^2$	3. Perimeter of elliptical section.	4. Relative volume of dim band before contraction $2a \times b^2$	Sphere with same circumference as ellipsoid: 5. Radius of sphere r	6. Volume of sphere $\frac{4}{3}\pi r^3$	7. Surface of sphere. $4\pi r^2$	8. Relative volume of dim band at end of contraction. $2r \times r^2$	9. Ratio of the volume of the dim band at end of contraction to that of the dim band before contraction Col 8 ÷ col 4	10. If light band and dim band are of equal length, contraction is to the following percentage of the original length— $\frac{2r \times 100}{4a}$
1	0·45	0·20	0·0755	2·13	0·0360	0·338	0·1610	1·440	0·0774	2·16	37·5
2	0·45	0·15	0·0425	2·02	0·0203	0·321	0·1382	1·295	0·0660	3·25	35·7
3	0·45	0·10	0·0188	1·906	0·0090	0·303	0·1165	1·155	0·0556	6·18	33·7
4	0·45	0·05	0·0047	1·83	0·00225	0·291	0·1030	1·065	0·0494	22·00	32·4

	11. Combined length of uncontracted light and dim band which can be completely absorbed by contracted dim band $2a \times$ col. 9	12. If light band is of such length that the whole muscle can be completely absorbed by the contracted dim band, the contraction will be to the following percentage of the original length— $\frac{2r \times 100}{\text{Col } 11}$	13. Increase in volume when ellipsoid becomes a sphere Col 6—col. 2	14. Ratio of the surface through which unit volume passes in muscle to unit volume passing through unit surface in the osmometer $\frac{5 \times (10000)^3}{19 \times (10000)^2} \div \frac{\text{col } 13}{\text{col } 7}$	15. Relative gradient of osmotic pressure in fibril compared with that in the osmometer Thickness of parchment paper ÷ r	16. Time in seconds for acid to attain equilibrium in muscle $\frac{14 \text{ days}}{\text{Col } 14 \times (\text{col } 15)^2}$	17. Time in seconds for osmotic pressure to produce maximal distension of sphere $\frac{3 \text{ days}}{\text{Col } 14 \times \text{col } 15}$	18. Total time in seconds for complete muscular contraction Col 16 + col 17
1	1·94	34·7	0·0855	44400	254	0·00043	0·0230	0·0234
2	2·93	21·9	0·0957	35700	268	0·00047	0·0272	0·0277
3	5·56	10·9	0·0977	31200	284	0·00048	0·0293	0·0298
4	19·80	2·94	0·0983	28500	296	0·00049	0·0307	0·0312

Sheep's blood was centrifugalised and the corpuscles washed once with 0·9-per-cent. sodium chloride. The corpuscles were diluted with an equal volume of distilled water and the resulting solution of hæmoglobin was placed in osmometers.

Solution outside osmometers	Maximum pressure	Time, in days, to maximum	Percentage of organic matter	Pressure per 1 per cent of organic matter
	mm			
600 c.c. water	108	2	14·8	7·3
600 c.c. water	100	2	14·6	6·85
600 c.c. 0·01 N lactic acid	248 } Average increase 153	8	14·5	17·2
600 c.c. 0·01 N lactic acid	266 }	7	14·0	19·0

This experiment illustrates how the anisotropous substance may swell as the result of an increase in osmotic pressure due to the formation of lactic acid.

Bernstein (2) states that the force of frog's muscle is about 600 grm. per square centimetre, which amounts to a pressure of 445 mm. of mercury. The percentage of protein in muscle is about 18, so this is equivalent to 24·8 mm. per 1 per cent. of protein, a pressure not much greater than that recorded above with a strength of acid which is less than that formed in muscle during contraction.

Macallum (8) and Macdonald (12) have shown that the inorganic constituents of muscle are mainly confined to the dim band. Some years ago I endeavoured to find out if the proteins were also mainly confined to the dim band. Crab's muscle was stained by immersion in dilute Millon's reagent, copper sulphate followed by alkali, and other protein reagents. In each case the dim band appeared to stain more deeply. The experiments were discontinued, as the appearances might have been due to the colour seeming darker because of the opacity of the dim band. If the proteins are unequally distributed less pressure per 1 per cent. of protein would suffice for muscular contraction.

Another way to calculate the osmotic force of muscular contraction is to regard the increase of pressure as due to the amount of lactic acid combined as an ionising salt with protein, the increase in pressure being considered to be due to the addition of the lactic ion to the protein.

The increase in pressure (153 mm.) is equivalent to a 0·009 normal solution. This added to the 0·01 normal solution, the amount of uncombined acid which should be equally distributed inside and outside the osmometer,

gives a concentration of 0·019 normal. To produce this concentration in the 25 c.c. of solution in the osmometer would require 5 c.c. of a decinormal solution of lactic acid. It would be difficult to show the disappearance of this amount from the 600 c.c. of N/100 solution outside the osmometer. In the last osmometer of the experiment 4·9 c.c. had disappeared, but such a close result is probably a coincidence.

If we wish to make a similar comparison in muscle we must calculate the relative volume of the anisotropic substance to the whole muscle in the uncontracted and contracted states. A disc of paper was divided into as many smaller discs as possible by a cork borer. These smaller discs collectively weighed 0·581 grm., and the residue weighed 0·224 grm. It was found, however, that there were certain larger pieces from the edge of the large disc that were too small to form small discs. If these pieces were excluded the weight of the residue was 0·153 grm. The larger the whole disc the less relatively is the waste at the edges, so that we can consider the weight of the smaller discs as between 79·2 and 72·2 per cent. of the whole; these weights represent the relative area of the combined smaller discs to the whole disc.

These figures give the relative volume of small cylinders inside a larger cylinder of the same length. The volume of an ellipsoid is two-thirds of a cylinder of the same length and diameter. Therefore the volume of the ellipsoids will be two-thirds of the above, namely, between 52·8 and 48·1 per cent. of the volume of the dim band. As the volume of the light band is approximately the same as that of the dim band, the ellipsoids will occupy half the above amount, that is between 26·4 and 24·1 per cent. of the uncontracted muscle.

In the contracted condition the whole of the muscle is contained in the dim band, and the ellipsoids become spheres. In the preceding paragraph we have calculated the relative volume of cylinders inside a larger cylinder, and since the volume of a sphere is two-thirds of the volume of a cylinder surrounding it, we see that the spheres occupy between 52·8 and 48·1 per cent. of the whole contracted muscle.

If as in the osmometer experiment the concentration of lactic acid in the isotropous substance remains as low as 0·01 normal, and the concentration in the whole muscle is equivalent to 0·025 normal (5), we can calculate the amount of lactic acid in the anisotropous substance. If the muscle is prevented from shortening, the concentration would be about 0·07 normal. If, on the other hand, the muscle is allowed to shorten, the concentration of lactic acid in the anisotropous substance would be about 0·04 normal. These figures are obtained by subtracting from the total concentration of lactic acid

the amount contained in the isotropous substance and concentrating the remainder in the anisotropous substance That is

$$[0\,025-0\,01\,(100-X)/100]\,.\,100/X,$$

where X is the percentage of the whole muscle formed by the anisotropic substance as calculated above

If we subtract the amount of uncombined lactic acid (0 01 normal) from the above we have left 0 06 normal and 0·03 normal respectively, as the amount of lactic acid as ionising salt in the anisotropic substance These would give osmotic pressures of 1018 mm. and 509 mm, both of which exceed the 445 mm which Bernstein states is the maximum required (2).

By the formation of a lactic-acid salt of protein a negative potential would be produced This is apparently the explanation of the negative potential produced when a muscle contracts (1, 3, 16, 19) The time relations of acid production are such that the acid can be considered as the cause of contraction (23)

There is one further point. It is claimed that if the contraction of muscle is due to changes in surface tension one explanation suffices for it and for amœboid movement Granting that amœboid movement is due to changes in surface tension, may it not be that the changes in surface tension are due to electrical charges, the result of acid production? Thus amœboid movement and muscular contraction may be related, but in a different way from that advocated by those who claim that muscular contraction is due to changes in tension at surfaces of separation. We do not yet know enough about unstriped muscle to suggest how its contraction is brought about.

The above outline gives a definite conception of muscular contraction which accounts for all the known facts If an equally tangible explanation could be furnished based on surface-tension changes, the two hypotheses might be compared one with another, and the two together might lead to a conception closer to the truth than can be arrived at from either hypothesis taken by itself

Summary of Conclusions

1. The contraction of striated muscle can be explained on the hypothesis that lactic acid is set free, and that this combines with protein to form a salt, with a consequent rise of osmotic pressure

2. Muscle can shorten by osmotic processes until its length is somewhere between 37 and 3 per cent of its original length (Table, columns 10 and 12).

3 The osmotic process can occur in frog's sartorius in less than 0 04 of a second (Table, column 18)

4 In order to determine whether all cases can be explained by this hypothesis it is necessary to have measurements of the structures concerned. Insect's muscle, for instance, should be the test as regards the rapidity of contraction

5 The amount of lactic acid formed during muscle contraction can cause sufficient rise in osmotic pressure to account for the force exerted by muscle contraction

6 The electrical changes in muscle can be explained by the formation of a protein salt of lactic acid

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On the Nutritive Conditions Determining the Growth of certain Fresh-water and Soil Protista

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[Plate 12]

It is well known that in ponds and lakes cycles of development occur, in which various kinds of animals and plants replace one another in succession, but the conditions are usually so complex that the succession rarely repeats itself with regularity from year to year, and it is impossible to assign, with any certainty, the successive phases to their determining causes. The same kind of cyclical development occurs in artificially made organic infusions, where bacteria, algæ, flagellates and ciliates replace one another in irregular sequence

The object of this paper is to indicate certain lines of experiment upon which it may be possible to attack this problem

Woodruff has contributed some data for studying the underlying causes of these successive events, and the work of numerous authors has added to our knowledge of the factors regulating the growth of algæ and diatoms Amongst these may be mentioned the work of Oswald Richter* on the nutrition of fresh-water algæ, and that of Miquel,† and more recently of Allen and Nelson,‡ on the culture of diatoms The work of these authors tends to show that, even in the case of algæ and diatoms in which nutrition appears to be holophytic, the presence of some organic matter in the culture medium is of great assistance to the growth of the organisms

The experiments with *Euglena viridis* were carried out with the object of investigating the nature of this organic matter which exerts a beneficial influence on the growth of apparently holophytic protista.

The method employed is to use a culture medium containing a constant proportion of the inorganic salts necessary for the nourishment of a holophytic organism, and to supply the organic matter in the form of

* Oswald Richter, 'Die Ernährung der Algen,' 1911

† Miquel, 'Le Diatomiste,' 1892.

‡ E. J Allen and E W Nelson, "On the Artificial Culture of Marine Plankton Organisms," 'Journ Marine Biol. Assoc.,' vol. 8, No 5, 1910

chemically pure organic compounds instead of the indefinite composition of an organic infusion

The method employed in the cultures of *Euglena viridis* has also been used to study the minute bacterial feeding flagellates living in the soil

Experiments with Euglena viridis

In culture experiments with *Euglena gracilis*, Zumstein* found that a much improved growth could be obtained if a little organic matter was added to the culture medium By increasing the amount of organic matter in the medium, he found that *Euglena gracilis* could be induced to change its mode of nutrition, living solely as a saprophyte Under these conditions the Euglena passed into an Astasia-like form, the chlorophyll disappearing and leaving only the colourless leucoplasts. When living saprophytically, the organism could thrive in the dark as well as in the light Zumstein found that the green coloration was reassumed if the Astasia form was brought back into a solution containing only a small amount of organic matter and kept in the light.

Treboux† was able to grow *Euglena gracilis* in solutions containing citric acid, but found that *Euglena viridis* could not be grown under these conditions Thus, there appears to be a marked physiological difference between these two species of Euglena, a fact which is emphasised by the earlier work of Klawkine‡ on *Euglena viridis*. His experiments showed that it was impossible to make *Euglena viridis* thrive well in the dark. When kept in the dark in a medium containing organic matter, the Euglenæ remained alive, but did not lose their chlorophyll or show a perceptible increase Our experiments with this species of Euglena have confirmed the results obtained by Klawkine, and show that *Euglena viridis* is not able to thrive in the absence of light, even when placed in the optimal culture medium and in the presence of suitable organic matter

It is thus evident that *Euglena viridis* is a more essentially holophytic organism than *Euglena gracilis*, a fact which tends to simplify the issue when we come to study the physiology of its nutrition

By appropriate methods, a culture of *Euglena viridis* has been kept in active growth in test-tubes by inoculation from tube to tube, for a period of about two years

* H. Zumstein, "Morphologie und Physiologie der *Euglena gracilis*," 'Pringsheim's Jahrbücher f wiss. Botanik,' vol 34, p. 419 (1899).

† O Treboux, "Organische Säuren als Kohlenstoffquelle bei Algen," 'Ber d. D. B Ges.,' vol. 23, p 432 (1905)

‡ W Klawkine, "Recherches biologiques sur l'*Astasia ocellata* et l'*Euglena viridis*," 'Ann des Sci Nat, Zool,' série 6, vol. 19, and série 7, vol. 1.

The medium used for growing these organisms has been a mixture of inorganic salts given by Miquel in his paper on the growth of diatoms.* To this medium, which contains all the elements necessary for the growth of a green plant, it has been found necessary to add some organic material in order to obtain an active growth of the organism. The composition of Miquel's fluid is as follows:—

Solution A.		Solution B.	
$MgSO_4$	10 grs.	Sodium phosphate	4 grs.
NaCl	10 "	Calcium chloride	4 "
Sodium sulphate	5 "	Hydrochloric acid	2 c.c.
Ammonium nitrate	1 "	Perchloride of iron, sat. sol.	2 "
Potassium nitrate.	2 "	Water	. 80 "
Sodium nitrate	2 "		
Potassium bromide	0·2 "		
Potassium iodide	0·1 "		
Water	. 100 "		

To make up the fluid, 40 c.c. of solution A and 10 c.c. of solution B are added to 500 c.c. of tap water, and the mixture is filtered.

It was necessary at first to determine the strength of Miquel's fluid best adapted for growing the Euglena. In a number of preliminary experiments it was found that the best growth could be obtained when 4 c.c. of the above Miquel solution were added to 6 c.c. of tap water, the organic matter being supplied by 1 c.c. of hay infusion. The experiment was performed by inoculating the tubes with a very small amount of the stock culture, introduced by means of a capillary pipette. The tubes were kept in diffuse daylight at room temperature; attempts to hasten the growth by incubation at 75–80° F. were unsuccessful, the Euglena dying, or at any rate failing to flourish at this temperature. In the absence of any organic infusion, the Euglena either failed to develop or else multiplied very slowly, and the fluid in the tube never became crowded with free-swimming organisms so as to appear opaque and green. The addition of 1 or ½ c.c. of hay infusion, on the other hand, caused a thick growth which, after the lapse of 10–14 days, filled the tube with myriads of free-swimming individuals, giving a totally different appearance to the condition seen in the tubes to which no organic matter had been added.

It was found, however, that the efficacy of the hay infusion varied very greatly according to the length of time during which bacterial growth had continued in it. Thus, a fresh hay infusion, after being sterilised, was found

* Dr. Miquel, 'Le Diatomiste,' No. 9, June, 1892.

to have a much feebler effect in stimulating growth than an infusion which had been kept for some weeks and in which bacteria had been allowed to multiply before it was sterilised

On the other hand, the same infusion, after being left for several months, lost much of its previous efficacy Similar results were obtained with other vegetable infusions

A typical experiment, showing the effect of the various dilutions of Miquel solution, and of the presence and absence of organic matter in the medium, may be seen in Table I

Table I —Cultures inoculated on April 21

Tube No	Composition of Medium		Growth on April 29	Growth on May 21
1	10 c c Miquel solution		None	None
2	8 c c " " + 2 c c distilled H_2O		"	"
3	6 c c " " + 4 c c " "		"	"
4	4 c c " " + 6 c c " "		"	"
5	2 c c " " + 8 c c " "		"	"
6	10 c c " "	+ 1 c c hay infusion	"	"
7	8 c c " " + 2 c c " "		"	"
8	6 c c " " + 4 c c " "		"	"
9	4 c c " " + 6 c c " "		Slight growth	"
10	2 c c " " + 8 c c " "		"	"
11	8 c c " " + 2 c c tap water		None	"
12	6 c c " " + 4 c c "		"	"
13	4 c c " " + 6 c c "		Slight growth	"
14	2 c c " " + 8 c c "		"	"
15	8 c c " " + 2 c c "	+ 1 c c hay infusion	None	"
16	6 c c " " + 4 c c "		Slight growth	"
17	4 c c " " + 6 c c "		Very strong growth	Very strong growth
18	2 c c " " + 8 c c "		Strong growth	Good growth

These experiments show that the best growth was obtained in tube No. 17, in which the culture medium consisted of 4 c c Miquel solution + 6 c.c. tap water, to which 1 c c. hay infusion was added, while in those tubes to which no organic matter was added, growth was either totally absent, or else a slight growth was observed in those tubes in which the proportions of Miquel and tap water approached the optimum

It must be noted that when tap water is replaced by distilled water, the growth is either prevented altogether or else is very slight, even when the necessary elements for growth are given in the Miquel and organic matter This can be seen in Table I in tubes Nos. 7–10, as compared with tubes Nos. 15–18 A similar result was noticed when cultures were made with Miquel fluid that had been made up with distilled water instead of tap water The improved growth in tap water might be due either to the

difference in osmotic pressure or to the influence of some constituent, organic or inorganic, in the tap water

An attempt was made to discover which of these was the determining influence. An artificial tap water was made up from an analysis of Thames water furnished us by Mr. W. W. Fisher. This contained:—

	Parts per 100,000
$NaCl$	2 8
$NaNO_3$	0 7
$MgSO_4$	1 4
$CaSO_4$	2 8
$CaCO_3$	22 3
SiO_2	1·0
Total solids	31 0

Somewhat conflicting results were obtained when this artificial Thames water was used to replace natural tap water· on the whole the growth obtained was not so good as when natural tap water was employed, but since it was possible to obtain quite good growths with the artificial medium, it must be concluded that the superiority of the media containing tap water is due to some slight alteration in the proportions of the inorganic constituents.

Having determined the optimum conditions for growth as far as the inorganic constituents are concerned, namely, 4 c c. of Miquel solution + 6 c c natural tap water, the question of the nature of the organic matter used by the Euglena was then taken up.

In the experiments given below, the ordinary method of inoculation by means of a capillary pipette was employed, and, in addition, another method which gives more rapid results. In this second method, an old culture tube, in which Euglena has been growing for a long period, is taken. In such a tube there is a ring of encysted Euglena adhering to the glass at the surface of the liquid. The liquid is poured away and the encysted Euglena, which is very firmly attached, may then be thoroughly washed with tap and distilled water. In this way a practically pure culture of Euglena may be obtained on adding the appropriate culture medium, though in no case has it been found possible to obtain a sterile culture free from bacterial contamination.

The following chemically pure substances were added to the "optimal Miquel" mixture and the tubes inoculated with Euglena, with the results subjoined.

1. *Dextrose*—

In media, in which 0·5–1 c.c. of a 1-per-cent solution of dextrose was added to 10 c.c. of the "optimal Miquel" solution, no growth of Euglena was observed, but in all cases there was a considerable growth of fungus, probably derived from spores from the stock tube of Euglena. It is probable that the great development of the fungus inhibited the growth of the Euglena, since a slight growth in the optimal Miquel solution was expected.

2. *Cane Sugar*—

The addition of 0·5–1 c.c. of a 1-per-cent solution of this substance to 10 c.c. of the "optimal Miquel" solution did not inhibit the growth of the Euglena to the same extent as the dextrose, but only rarely and after a long period did any noticeable growth appear. The growth of fungus in these tubes was either absent or very slight.

3. *Tartaric Acid*—

The addition of 1 c.c. of a 1-per-cent. solution had a purely negative effect, no growth of Euglena but a strong growth of fungus being observed.

It is evident from these results that the stimulating element in the organic infusion is not in the nature of a carbohydrate.

4. *Peptone*—

The addition of 1 c.c. of a 1-per-cent solution of peptone to the medium invariably gave rise to a very strong bacterial growth, the bacteria being no doubt introduced with the Euglena, on inoculation. Under these conditions the Euglena scarcely developed at all, although it is not entirely killed off, a slight ring appearing at the top of the fluid.

5. *Amido-acids**—

Tyrosin.—As this compound is very insoluble in water, a saturated solution was made up in distilled water. The saturated solution when cold contains the salt in the proportion of 1 in 2400 of water.

In the earlier experiments 1 or 2 c.c. of the above solution were added to 10 c.c. of the "optimal Miquel" mixture. A very strong growth was obtained in this medium, indeed superior to that obtained by means of the addition of a natural organic infusion. The marked difference in the growth of the Euglena in a tube containing this minute trace of tyrosin (1 : 24,000) as compared with a culture in a medium free from organic matter, may be seen in the photograph (fig. 1).

* A number of experiments have been made by Loew, Bokorny, and others on the growth of algæ in amido and fatty acids. A full literature of this work will be found in Oswald Richter's 'Die Ernährung der Algen,' 1911.

In cultures containing 1–2 c c of the tyrosin solution, it was found that after a period of about six to eight weeks the Euglena ceased its active growth and became encysted upon the walls of the tube and especially round the surface. For example, a tube containing 4 c c Miquel solution, 6 c.c tap water, and 1 c.c. tyrosin solution, was inoculated with Euglena on November 25 By December 3 there was a noticeable growth, which became very thick by December 30 By January 30 all free-swimming forms had disappeared, and nothing remained but a ring of encysted

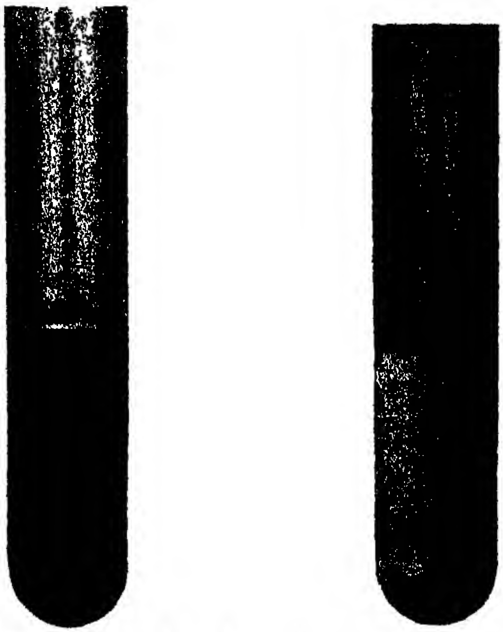


FIG 1.—Photograph to show the Growth of Euglena in a Tube (B), containing the optimal Miquel mixture, to which tyrosin solution was added, as described.

Tube A shows the slight growth in a control culture containing the optimal Miquel solution, but with no organic solution.

Photograph shows a 10-days growth

Euglena. It was found that by replenishing the culture medium in this tube, the growth of the Euglena could at once be revived within two days after replenishment a thick growth of free-swimming forms was obtained

This suggested that the tyrosin was used up after a certain period To test this hypothesis, cultures were made in the optimal Miquel mixture, to which tyrosin was added in the solid form, so that as soon as the dissolved tyrosin was used up, fresh tyrosin might go into solution. Cultures grown in this medium showed a very rapid growth of Euglena during the first fortnight or three weeks, but after that the increased development of

bacteria in the culture usually interferes with the Euglena growth. The following culture may be regarded as typical of the growth of Euglena in a medium of this nature:—

Cultures with Tyrosin Media, inoculated February 2.

Composition of tube	Growth on Feb 5.	Growth on Feb 18.
4 c.c Miquel tap + 6 c c tap water + solid tyrosin	Very strong Euglena growth	Euglena dead or encysted; numerous bacteria
4 c c Miquel tap + 6 c c tap water + 1 c c tyrosin solution	Slight growth of Euglena	Very strong Euglena growth; very few bacteria

To avoid the excessive growth of bacteria in the tyrosin and at the same time to ensure the continuous supply of tyrosin, the following culture method was devised The Euglena was grown in a tube containing the optimal Miquel mixture alone, and the trace of tyrosin was supplied from another tube containing a saturated solution of this substance connected by means of a capillary tube with the Euglena culture. In this way the culture medium is continually supplied with traces of tyrosin solution, but the diffusion is too slow to cause an excess of tyrosin in the tube containing the Euglena. It was found that by this method a strong growth of Euglena could gradually be obtained, nearly free from the bacteria and minute flagellates which always appeared in cultures to which solid tyrosin was added.

Of all the culture media employed the thickest and most successful growths of Euglena have been obtained with optimal Miquel mixture to which tyrosin is added.

Glycocoll—Cultures were made in optimal Miquel mixture to which was added 1 c c of 1-per-cent. solution of glycocoll These cultures invariably gave a strong growth of bacteria and, at first, a greatly retarded growth of Euglena, though subsequently the Euglena increased In no case did these cultures compare in strength of growth with the cultures in tyrosin media. It is probable that this retardation was due to the bacterial growth, and this subject will be dealt with in the latter part of the paper.

Alanine.—1 c.c. of a 1-per-cent. alanine solution was added to the Miquel mixture as usual. The cultures invariably gave a very strong bacterial growth, and very frequently a bacillus producing a vivid apple-green coloration appeared. This green colouring matter was shown not to be chlorophyll, as it was developed more rapidly and to a greater degree in the dark than in the light. At first, as in glycocoll, the Euglena failed to multiply, though after a long period, viz. about three weeks, tubes inoculated

with a ring of encysted forms, as described above, produced a considerable growth.

The great superiority of the tyrosin solutions over the solutions of glycocoll and alanine was very marked It was at first thought possible that this was due to the presence of the benzene ring in the tyrosin, especially since alanine is similar in composition to tyrosin, except that in the former substance the oxyphenyl ring is absent

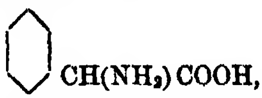
$CH_3.CH(NH_2)\,COOH$

Alanine

Tyrosin

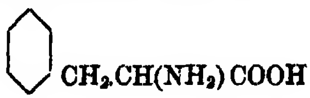
With a view to testing this hypothesis, the phenyl compounds of alanine and glycocoll were employed in the culture media.

In media containing phenyl glycocoll,



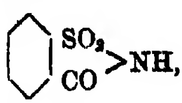
it was found that no growth took place, even the development of the bacteria being prevented

But, in the media containing phenylalanine,



a very strong growth of Euglena was produced, bacterial growth being at first slight, but increasing after some time. Since this compound resembles tyrosin in being very insoluble, it was added to the media in a solid form

Attempts to grow Euglena in saccharin,



showed that this substance prohibited all growth of the organism

The negative results obtained with phenyl glycocoll and with saccharin showed that, at any rate, the mere presence of the benzene ring was not the essential factor for the growth of the Euglena.

Since the substances that are most successful for the propagation of Euglena, namely, tyrosin and phenylalanine, are only very slightly soluble, so that exceedingly weak solutions are used, and since, on this account, bacterial growth in these solutions is very slight compared with that which occurs in the stronger solutions of alanine and glycin, it seemed possible

that the strong growth of the Euglena might be connected with the slight bacterial growth.

To test this hypothesis, lesser amounts of alanine and glycocoll were added to the Miquel mixture.

From 0·2 c.c. to 0·5 c.c. were added to 10 c.c. of the optimal Miquel mixture. In cultures started from a ring of encysted forms it was found possible to obtain extremely good growths of Euglena by this means, the bacterial growth being very much lessened.

It is thus obvious that the Euglena can use alanine and glycocoll, as well as tyrosin and phenylalanine, provided its growth is not inhibited by the rapid development of bacteria, such as always takes place in the glycocoll and alanine solutions when they are too strong. This result is of importance as indicating that the amido-acids are used as such by the Euglena, and not after being decomposed by bacterial growth. Thus, attempts made to grow Euglena in tubes containing an alanine medium in which bacterial decomposition had proceeded for a long time were entirely unsuccessful.

It is interesting to notice in this connection the fact mentioned above that, in the case of tyrosin, the addition of the solid substance to the tubes causes a considerable bacterial growth, which, after about three weeks, was sufficient to inhibit the proper growth of the Euglena. Nencki* has shown that, under the influence of anaerobic bacteria, tyrosin is converted into oxyphenyl-propionic acid,

and it is probable that, under the aerobic conditions met with in the culture tubes, further decomposition into oxyphenylacetic acid and phenol takes place.

Decomposition along similar lines occurs when phenylalanine is subjected to bacterial growth, phenylpropionic acid and phenylacetic acid being formed. Thus, when tyrosin and phenylalanine are added in the solid condition, their solutions are sufficiently strong to allow a growth of bacteria, which decompose them into phenol derivatives that are harmful to the Euglena growth.

We may suppose that, in the same way, harmful products are produced by bacterial action on alanine and glycocoll, so that the Euglena is prevented from developing in the solutions of a strength adapted to the growth of bacteria.

Other nitrogenous compounds have been tried, *e.g.* urea, uric acid, and

* Nencki, 'Ber. d. Deutsch. Chem. Gesellsch.,' 1874, p. 1593.

allantoin. All these substances gave negative results, and no growth of Euglena could be obtained in optimal Miquel mixture to which these substances were added

Thus, no substances other than compounds of the amido-acid type, have been found suitable for stimulating the growth of the Euglena.

If we enquire into the part played by the amido acids in the nutrition of Euglena, it may first be noted that the Euglena is obtaining the greater part of its nutriment from the CO_2 of the air and from the mineral substances in the Miquel mixture. This was readily proved by keeping a control tube, containing the optimal Miquel solution to which tyrosin had been added, in the dark, in which case the growth of the Euglena was at once arrested (fig 2).

It must also be pointed out that the amount of amido-acid present in the optimal culture medium is exceedingly minute, *e g* in the case of tyrosin

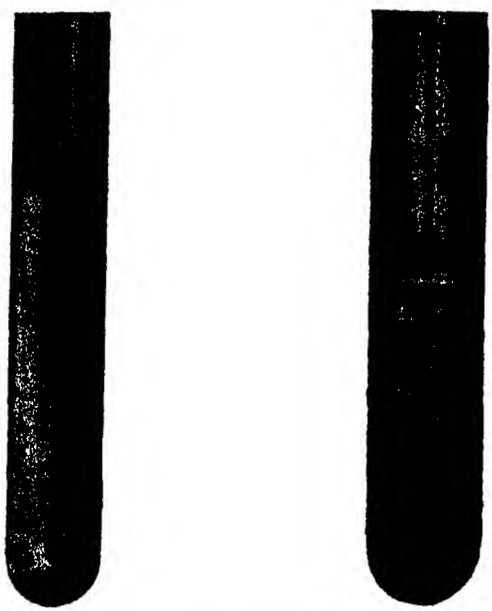


FIG. 2 —Photograph showing the Growth of Euglena in Miquel Mixture and Tyrosin, in the dark (tube C), and in the light (tube D)

Photographed three weeks after inoculation.

solution the amount of the salt is only 1 part in 24,000 of liquid It is very remarkable that so minute a trace of organic matter can make so great a difference in the rapidity of growth and reproduction in an organism as shown in the first photograph (fig 1) It would appear that the organic substance acts more as a stimulant than as a direct source of nutriment.

The facts observed in the culture of Euglena may be summarised as follows:—

(*a*) In solutions containing no organic matter, the Euglena increases very slowly

(*b*) By the addition of a trace of organic infusion to the solution of inorganic salts, a good growth of Euglena can often be obtained

(*c*) The efficacy of the natural organic infusion in stimulating the growth was very variable

(*d*) Minute traces of amido-acids added to the inorganic solution had a remarkable effect in stimulating the growth of the Euglena

(*e*) Stronger solutions of amido-acids were less successful owing to the rapid development of bacteria in the medium.

(*f*) The Euglena does not appear to live saprophytically on the amido-acid, since it cannot be made to thrive in the absence of light

2 *Experiments with Soil Protozoa.**

The method of growing Protozoa in solutions containing a mixture of Miquel in tap water to which various organic compounds are added was also applied with a view to studying the protozoal fauna of various soils

The mode of procedure was similar to that employed in the experiments on Euglena The cultures were made in sterilised test-tubes to which the optimal Miquel solution was added, the solutions also being carefully sterilised Various organic solutions were added to the various tubes, which were inoculated by adding a small amount of soil to each tube. This method was found to be particularly suited to the culture of the minute soil flagellates, more especially *Prowazekia terricola* described by Martin.†

The following Table shows a typical series of cultures conducted as described above:—

* See Dr Russel and Dr Hutchison, "On the Effect of Partial Sterilisation of Soil on the Production of Plant Food," 'Journ. Agric. Sci.,' vol. 3, part 2 (1909); also Goodey, 'Roy. Soc. Proc.,' B, vol. 84, p 165 (1911)

† C H Martin, 'Zool Anzeiger,' vol 41, No 10 (1913). A flagellate monad, similar to that described by C H Martin ('Roy Soc. Proc.,' B, vol 85, 1912), was found in small numbers in our cultures.

Composition	Inoculation, March 8	Observations on March 13
4 c c Miquel tap + 6 c c tap—		
1 + 1 c c cane sugar solution	Controls Not inoculated	Few bacteria only
2 + solid tyrosin		Sterile
3 + solid phenylalanine		Sterile
4 + 1 c c cane sugar	Inoculated with stale manure	A few soil flagellates Some ciliates
5 + solid tyrosin		Very large numbers of soil flagellates and of soil amœbæ
6 + solid phenylalanine		Large numbers of flagellates; a few ciliates
7 + 1 c c cane sugar	Inoculated with leaf mould	A few soil flagellates
8 + solid tyrosin		Very large numbers of flagellates
9 + solid phenylalanine		Large numbers of flagellates.
10 + 1 c c cane sugar	Inoculated with ploughed soil	Very few flagellates
11 + solid tyrosin		No flagellates
12 + solid phenylalanine		Very few flagellates
13 + 1 c c cane sugar	Inoculated with soil under grass land.	No flagellates
14. + solid tyrosin		Very few flagellates
15 + solid phenylalanine		Fair number of flagellates

The above Table illustrates the fact that while the minute soil flagellates thrive best in tyrosin or in phenylalanine solutions, yet they are able to develop in solutions containing cane sugar. Cultures were made with the object of ascertaining the effect of various other organic substances on the growth of the flagellates The flagellates in these cultures were derived for the most part from a stock tube of Euglena culture in tyrosin, in which Prowazekia was also very abundant. The following list embodies the results obtained with various organic substances Save where otherwise mentioned, the organic compounds were added in the proportion of 1 c c of a 1-per-cent solution to 10 c.c of the optimal Miquel mixture in tap water

	Growth of the flagellates
Peptone	Good growth
Tyrosin (OH $C_6H_5.CH_2.CH(NH_2)COOH$)	Very strong growth
Tyrosin (added solid)	Optimum growth.
Phenylalanine, $C_6H_5.CH_2.CH(NH_2)COOH$ (added solid)	Strong growth.
Alanine $CH_3.CH(NH_2)COOH$ (0 5 c c. of 1-per-cent solution)	Fair growth
Glycocoll, $CH_2(NH_2)COOH$	Fair growth
Phenylglycocoll, $C_6H_5.CH(NH_2)COOH$	No growth
Allantoin	No growth
Saccharin	No growth
Cane sugar	Good growth
Tartaric acid	No growth

These cultures showed that the soil flagellates were able to grow in tubes containing a large variety of organic substances, in many of which Euglena is

unable to thrive This is the result of the holozoic mode of nutrition of the flagellates, which feed greedily on the bacteria in the culture and are always to be found in greatest abundance in the bacterial scum at the surface

The development of the soil flagellates in the culture is evidently dependent upon the bacterial flora in the tube. In the tyrosin media the bacterial growth reaches its most favourable degree for the development of the flagellates. In the case of cultures in media containing alanine it is frequently found that the flagellates fail to attain their maximum growth, being probably swamped by the excessive numbers of bacteria.

In order to discover whether the Miquel salts were necessary for the growth of the soil flagellates, a culture medium was made up by adding solid tyrosin to 10 c.c of tap-water, and this was inoculated with a strong culture of *Prowazekia terricola* This culture entirely failed to develop, remaining almost entirely free even of bacteria, which were evidently unable to develop satisfactorily in the absence of the salts of the Miquel solution.

The Prowazekia were observed to flourish in cultures containing very varied types of bacteria. In order to discover whether the flagellates exercised any selective faculty when feeding upon the bacteria, a number of smear preparations were made from different cultures. The films were fixed with corrosive acetic or with osmic vapour, and were stained with iron hæmatoxylin These preparations showed that bacteria of all the types surrounding the flagellates were ingested in quite a promiscuous manner (see Plate 12).

The cultures inoculated with various soils, both in the test-tubes and in drop cultures which were also made, show the enormous abundance and wide distribution of these minute flagellates as compared with other soil protozoa.

Although ciliates and amœbæ often fail to appear in tubes inoculated with a very small quantity of soil, yet all the types of soil that have been tried have yielded at least some Prowazekia when inoculated into the appropriate culture media. The organism has also been found in tap water and in water from an open-air tank.

The very rapid increase of these minute flagellates is also very noticeable. Under the optimum culture conditions it has been found possible to obtain a strong growth of the flagellates within 48 hours of the time of inoculation. On the other hand the larger protozoa, such as the ciliates, do not become even noticeable in the tubes until a week or so has elapsed.

The great abundance and wide distribution of the minute flagellates, taken in conjunction with their rapid powers of increase, suggest that in all probability they are of much greater importance than the larger soil protozoa as a factor in the destruction of soil bacteria.

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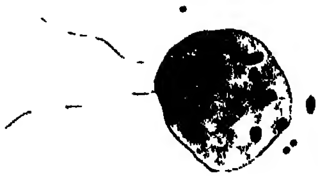

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The ease with which Prowazekia can be grown in culture media containing tyrosin suggests the possibility of investigating its distribution in various types of soil Experiments in this direction are at present very incomplete, but as far as they go they tend to show that rich manure soil or leaf mould contains a considerably greater number of the minute flagellates than less rich soils. This is well seen in Table II (Compare Nos. 4–9 with Nos 10–15.)

In summing up the points observed in the cultures of soil flagellates we notice the following facts —

(*a*) As compared with Euglena they are able to live in cultures to which organic compounds of very varying natures have been added

(*b*) This comparative impartiality is the result of the holozoic mode of nutrition, the development of the flagellates being absolutely dependent on the bacterial growth

(*c*) The presence of the Miquel salts in the solution is necessary for the growth of the soil flagellates and for the proper development of the bacteria upon which they feed.

(*d*) The flagellates can feed upon a variety of different types of bacteria.

DESCRIPTION OF PLATE.

Soil Flagellates from a Culture containing a Mixed Bacterial Flora, showing various Types of Ingested Bacteria. × 2000

Figs. 1–2 —Two individuals containing ingested bacilli.

„ 3–6 —Individuals containing cocci of two kinds

„ 7–8.—Two individuals containing partially digested bacteria

The Validity of the Microchemical Test for the Oxygen Place in Tissues.

By Alan N Drury, B A, Shuttleworth Student of Gonville and Caius College

(Communicated, with a Note, by W B Hardy, F R S Received April 25,—Read June 18, 1914)

(From the Physiological Laboratory, Cambridge)

In the last few years endeavours have been made to locate precisely certain reactions known to occur in the living cell, and much work has been done especially on the reduction place, the oxygen place, and the position of oxidases and peroxidases

The reaction relied upon to indicate the position is always one involving a colour change, as an example, Unna's* method of fixing the oxygen place in the cell may be chosen He uses for this purpose a solution of rongalit white,† which is a solution of the leucobase of methylene blue kept in a state of reduction by excess of rongalit, an adsorption product of formaldehyde with sodium sulphite. This solution is not affected by air or by light, but is, according to Unna, a test for active oxygen. He places the section in a solution of rongalit white for one minute, then washes in a large volume of water, when, the reducer having been washed away, the tissue is able to show its ability to oxidise, and all the tissue elements which are capable of effecting an oxidation are blued owing to the oxidation of the methylene white to methylene blue.

Unna has noticed that it is possible to abolish the staining of the oxygen place by rongalit white, by the action of heat, neutral salts, alcohol, phenol, and other protoplasmic poisons, while an ordinary nuclear stain is not thus affected. Also the intensity of staining can be altered by previous treatment with alcohol, formalin, or gum, while the action of a nuclear stain is unaltered. He, therefore, asserts that the stained portions of the tissue are the oxygen places.

It is to be noticed that these comparisons are made between a very complex mixture, namely, rongalit white, and a simple solution of a dye, so that they are of little value unless controlled by an exact determination of the influence of the constituents of rongalit white on the absorption process

* "Die Reduktionsorte und Sauerstofforte des tierischen Gewebes," P. G Unna, 'Arch für Mikr Anat,' vol. 78 (1911).

† "Zur Chemie der Haut. 6.—Hautreagentien," Unna and Golodetz, 'Monatshefte f. Prakt. Dermat,' vol 50, p. 451 (1910).

Unna claims also that methyl green picks out the oxygen foci of the cell, and on experiments with this and other dyes he bases a claim that staining is controlled by the oxidising or reducing properties of the substances exposed to the stain

Neither experiments nor conclusions are above criticism. The problem of dyeing is to discover the conditions which control the condensation of the dye on to a surface separating a solid from a fluid The presence of oxygen must affect the process, since, like any other chemical substance, the oxygen will contribute to the chemical, electrical, and mechanical potentials which determine the degree of condensation, the purpose of this paper, however, is limited to the proof by exact physical experiment that there is no special connection between the presence of oxygen and the process of dyeing

Experiments with Silk.

The first substance tried was silk, and the procedure was as follows A tube was arranged which had a three-way tube joined to it, down one of which nitrogen gas could be passed, down the next rongalit white solution, and down the third nitrogen water, so that they could be changed one to another by closing or opening taps

The rongalit white was freed from oxygen by passing a stream of nitrogen through it for about six hours, the nitrogen itself having been passed through potash bulbs and bottles containing alkaline pyrogallate, to ensure that it did not contain oxygen

The water was boiled for 10 minutes and was cooled, while a stream of nitrogen passed through it. The silk was fixed in the tube and some nitrogen water was passed over it, the water was then turned off and nitrogen gas was passed over it; after an hour or so the gas was turned off and water again allowed to flow over, this again being replaced by nitrogen gas. Such a procedure was carried on for 5–7 hours The rongalit white was then allowed to flow into the tube, and, after it had covered the silk for 1–2 minutes, the excess of rongalit was removed by a stream of nitrogen water flowing for 5 minutes. The tube was then opened to the air In no case was any blueing observed until the tube was exposed to the air

The experiment divides itself into three stages —

1. The exposure of the silk to the rongalit white solution (under nitrogen)
2. The washing off of the excess of the rongalit still under nitrogen.

No signs of blueing were observed in these two stages

3. The exposure of the silk to the air, when the methylene white is oxidised to methylene blue.

The staining, as it is usually understood, namely, the condensation of the

solute on to a surface, takes place in Stage 1, so that it is obvious that this stage can occur in the absence of free oxygen—that is, of oxygen other than what may be still clinging to the silk surface The final effect, namely, the development of colour, is no part of the staining process, and is not an indication that the surface of the silk has any special affinity for oxygen, or is a place where oxidation is taking place

Such experiments as this, however, and similar ones, with gelatine, agar, and the gel of silicic acid, leave the fundamental proposition, which seems to be the basis of Unna's work, untouched, namely, that the condensation of a basic substance with a high avidity for oxygen, such as the reduction product, the leucobase of methylene blue, or of the fully oxidised coloured body methylene blue, occurs either where oxidation is taking place or where there is a condensation of oxygen To disprove this contention it is necessary to show that condensation may occur on to the surface of a body already fully oxidised, and completely freed from the film of condensed oxygen, which adheres so tenaciously to solid surfaces which have been exposed to air As is well known, a solution of a basic dye filtered through a layer of sand is decolorised, the dye being condensed on to the sand particles, sand, therefore, was chosen

Experiments with Sand

The procedure was as follows:—About 2 inches of sand were packed in a small combustion tube, having a pad of asbestos at one end to prevent the sand from washing through, when the various solutions were passed through. Hydrogen, prepared in a Kipp apparatus, was washed in water to remove acid, passed through a strong solution of alkaline pyrogallate to remove oxygen or traces of acid still remaining, and finally passed through a long tower of calcium chloride to remove water

The apparatus used was similar to that used for silk, save that the three-way tube was fixed on to a combustion tube which contained the sand. The sand was heated to redness in a furnace, and the purified hydrogen passed over it while it was in this condition The heating was followed by cooling, and this by heating again, still, of course, in a stream of hydrogen, and this was followed by cooling once more in a stream of hydrogen. These series of operations were carried on for three to four hours, so as completely to burn off the oxygen.* After the sand had cooled in the atmosphere of hydrogen, rongalit white solution, freed from oxygen by passing nitrogen gas through it for six hours, was allowed to flow in. It was allowed to stay

* Compare "Contact Electricity," F M Spiers, 'Phil. Mag,' 5th ser., vol. 49, Part 1 (1900).

in the tube for one or two minutes, after which it was washed through by a stream of nitrogen water

The sand was then treated in one of two ways—it was either kept in the tube, the two ends of which were open to the air, or it was washed through by the nitrogen water on to filter paper. The object of the second procedure was to eliminate the possibility that it might be only the solution of rongalit white trapped between the sand grains that underwent oxidation, the methylene blue so produced being taken up by the sand If any such trapped solution exists, it is rapidly taken up by the filter paper

The results which the first method gave are interesting. It was noticed that no blueing occurs for a considerable time, although the tube has been full of air That is to say, the hydrogen which has replaced the oxygen on the surface of the sand continues in possession of that surface for some time after it is brought into the presence of oxygen A similar condition is met with in the case of iron, if the oxygen which is normally condensed on the surface of the iron is completely replaced by a layer of hydrogen, the potential difference between the iron and another metal plate is changed When the iron is brought into the presence of air the reversion to the original potential difference is very slow, thus showing that the hydrogen is only slowly displaced from the surface of the iron.*

Details of an Experiment

3 10. Sand heated to redness and hydrogen passed through.

3.40 Allowed to cool in a stream of hydrogen.

3 50. Again heated to redness in hydrogen stream

5 0 Allowed to cool in stream of hydrogen

5 15 Rongalit white solution passed through and allowed to remain in contact with the sand for one minute There was no appearance of any colour at all.† Nitrogen water was then passed through for three minutes, again there was no sign of colour in the sand The nitrogen water was then driven through by a stream of nitrogen gas

5 25. Tube opened to the air and shaken to disturb the gas inside the tube.

5.40. No colour

6.0. No colour.

6 30. Slight blue colour beginning to develop

From this time onwards the colour gradually developed and deepened, till at 12.0 it had become dark blue.

* *Loc. cit.*, p. 2.

† Ordinary sand when placed in rongalit solution turns a green-blue colour.

The method of spreading the sand on to dry filter paper gave similar results.

1.30. Sand heated to redness, stream of hydrogen passed over
2 0 Sand allowed to cool in hydrogen
2 10 Sand again heated in hydrogen.
2 40. Sand allowed to cool in hydrogen
2 50 Sand again heated in hydrogen
3 20 Sand allowed to cool in hydrogen
3.30. Rongalit white solution allowed to flow in and to remain in tube for one minute This is followed by a stream of nitrogen water There was no sign of colour during these two stages
3 45 Sand washed out on to filter paper, no colour.
4 0 Slight blue-green tinge through the sand
5 15. Sand had become much deeper blue, the intensity of which continued to increase for some hours

This experiment agrees with the former in every particular, except in the time taken for the first appearance of the blue colour This is a difference rather to be expected than otherwise, as in the washing out on to the filter paper the surface would be very much disturbed, and consequently the condensed hydrogen would be more rapidly displaced.

That the oxygen-free surface clings tenaciously to the rongalit white condensed on to it appears from an experiment carried out in the same way as the preceding The sand was freed from oxygen, and then the oxygen-free rongalit-white solution was passed over for one minute After this the nitrogen water was allowed to flow through until the water as it emerged contained only very minute traces of methylene white. The sand inside the tube was then exposed to air on filter paper, and it developed a quite appreciable blue colour. There is thus no doubt that sand freed entirely from oxygen not only condenses methylene white on to its surface, but also holds it with a certain degree of pertinacity.

Experiments on the Effects of the Gases condensed on the Surface on the Condensation of Methylene Blue

1 *Oxygen* —A small combustion tube was filled with two inches of sand, having at one end a plug of asbestos to prevent the sand from being moved by the solution as it passed through.

Through this tube a solution of methylene blue was allowed to flow by gravity. The effluent was at first colourless, but with lapse of time as the

sand became saturated to the dye the colour increased to an intensity indistinguishable from that of the solution sent in

2 *Hydrogen*—The sand was alternately heated and cooled in an atmosphere of hydrogen gas completely to remove the oxygen. When it had cooled down in the hydrogen gas a solution of methylene blue of the same strength as was used above, but which had been made up in carefully boiled water, and had had nitrogen gas passed through it for six hours, was allowed to flow through by gravity as before Samples of equal volumes were collected at the other end, and these showed an exactly similar graduation from colourless to the colour of the solution sent in.

If there was any quantitative difference in the amount of condensation of methylene blue in the two cases, it could not be shown by such an experiment This point will be dealt with later.

It will be noticed here that sand whose surface is freed from oxygen and occupied by a film of condensed hydrogen will condense methylene blue from a solution so as completely to decolorise it

In the face of these results it is difficult to lay more importance on the results obtained by Unna than that he is merely picking out the basophile portions of the tissue with the rongalit white, the staining being modified, as might be expected, by the presence of the alkaline reducing substance, rongalit

The methylene white and the rongalit would both saturate the tissue with which they are brought in contact, but the rongalit is more easily dislodged than the methylene white, so that the basophile parts of the cell to which the latter clings would show a blue coloration owing to the oxidation of the methylene white to methylene blue

Quantitative Experiments on the Effect of Oxygen upon the Amount of Methylene Blue condensed on to Sand

The preceding experiments show that the presence of oxygen at a surface is not necessary for the condensation of either the highly oxidisable leucobase, or of methylene blue. We now proceed to the further question, how far does the film of condensed oxygen favour condensation or the reverse? It will be seen that it actually lessens condensation of methylene blue.

The following experiment was made. A solution of methylene blue was made and divided into two parts, one of which was freed from oxygen by passing nitrogen through it

Two combustion tubes were filled with similar lengths of sand, and were heated in a furnace. Over one was passed a stream of hydrogen to replace the oxygen, and over the other a stream of air was passed. The tubes were

heated for three hours and one hour respectively and were allowed to cool in their respective gases. The sand was then turned into the methylene blue, the hydrogen sand into the nitrogen methylene blue, the air sand into the air methylene blue.

After they had remained in the methylene blue for equal periods of time, the solution was decanted off, and the sand carefully dried, the intensity of blueing of the sand showing a very appreciable difference even to the eye The solutions of methylene blue were compared by means of the colorimeter with the original solution The sand was heated strongly to vaporise the methylene blue on its surface, and was then weighed

Account of an Experiment.—Sand was heated in a hydrogen atmosphere for three hours, and cooled in an atmosphere of that gas, it was then put into nitrogen methylene blue solution for five minutes The sand was decanted off and was compared with the original methylene blue solution by means of the colorimeter. The sand was dried, heated to volatilise the methylene blue, and weighed.

A similar quantity of sand was likewise heated in a stream of air for three hours, cooled and put into ordinary methylene blue solution of the same strength for five minutes, decanted off and compared with the original solution by means of the colorimeter The sand was dried, heated, and weighed. The same volume of solution was used in both cases:—

	Wt	Sol	Orig sol
A.—Sand heated in hydrogen .	1·75	0·8	0·5
B.— „ „ air.	1·03	0·75	0·5

The result can be represented as columns of methylene blue solutions on the same base and containing the same amount of dye

Original solution . .	5
Solution A	7·5
Solution B	12·5

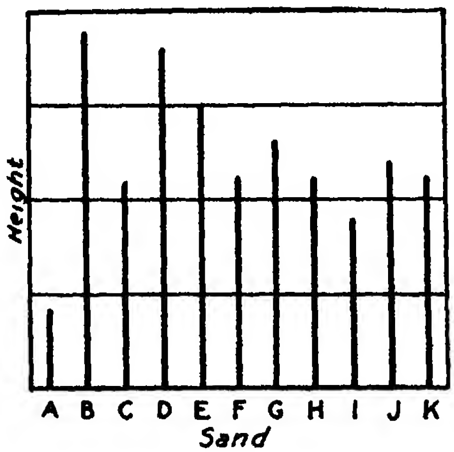
This experiment shows that the gas condensed on the surface plays an important part in the depth of staining which the surface undergoes. An explanation is, perhaps, to be found in the alteration of the electrical potential of the surface

The Effect of Certain Chemical Substances on the Amount of Methylene Blue Condensation on Sand.

In the following experiments the sand was shaken up with the substance to be tested, and washed in water The methylene blue solution was then

added and shaken for one minute, allowed to settle for one minute, after which it was decanted off, the solution was then compared with a sample of the original solution by means of the colorimeter The same volume of the methylene blue solution was used in every case, the sand was finally dried, heated to volatilise the methylene blue condensed on the surface, and weighed The following results were obtained.

The ordinates represent the heights of columns of methylene blue solutions



on the same base and containing the same amount of methylene blue, and consequently the relative desaturation of the original solution

A The height of the column of the original methylene blue solution with which the various solutions were compared.
B Ordinary sand.
C Sand previously treated with gum
D. " " chloroform
E " " formalin
F " " rongalit
G. " " mercuric chloride
H " " soap.
I. " " octyl alcohol
J. " " caprylic acid
K. " " *p*-cymol

These results show, as might be expected from theory, that the previous treatment of the sand has a large influence on the amount of methylene blue condensed on the surface

Summary.

Experiments were made which prove that the results obtained by Unna with rongalit white do not justify his assumption that it is a specific stain for the oxygen place in tissues, consequently his theory of staining by oxidation and reduction is not proven Further experiments were performed to find the effect of the gases condensed on to a surface upon the depth of staining.

Altering a surface by preliminary treatment with various chemical substances also has a marked effect upon the subsequent condensation of methylene blue

I should like to express my thanks to Mr Hardy for help and criticism The expenses of this research were defrayed by a grant from the Thruston Memorial Fund, Gonville and Caius College, Cambridge

[*Note by W B Hardy*—The fundamental uncertainty in all microchemical tests, and perhaps especially in those for oxidation places, may be put as follows —It is easy, as the writer found many years ago, to get discriminating colour reactions in sections and unfixed cells with oxidisable bodies such as Würster's tetra-substance, which is a singularly delicate test for what is called active oxygen—that is to say, for oxygen whose chemical potential is raised above that of atmospheric oxygen by, *e g*, ionisation or the formation of peroxide When oxidised it becomes a vivid purple and the purple reaction is given very definitely by, *e g*, the basophile granules of leucocytes when the cells are exposed to a trace of the substance But the tetra-substance is itself unfortunately a basic substance and would therefore be condensed from solution by the basophile granule in the ordinary process of staining

There appear to be three possibilities, and experiment seems unable to choose between them —

1 That the basophile granule is in fact a region where active oxygen is produced, *e g*, in the course of some local oxidation process

2 That the tetra-substance is oxidised indiscriminately about the section during manipulation, in the course of which it is probably exposed to the combined influence of evaporation and light,* and that it is subsequently condensed on to the basophile granules by a simple staining process.

3 That in the process of condensation of an oxidisable body by surface energy its chemical potential is raised, so that oxidation, which would not otherwise occur in the presence of atmospheric oxygen, actually does occur It must be noted that the condensation is due solely to surface forces,

* D'Arcy and Hardy, 'Journal of Physiology,' vol. 17, p. 390 (1894).

and has nothing to do with the particular surface being one specially prone to oxidation or reduction In this connection I am not forgetting that, when once completely condensed, the chemical potential of the oxidisable substance is no higher than (it is in fact identical with) what it is in the solution, but many instances show that during transition the molecules are under stresses which may find relief in exceptional chemical activity. For instance, the exceptional electrical and chemical properties of gases entering or leaving the surface of platinum; the high chemical potential of condensing oxygen in Prof Bone's experiments on surface combustion, while by contrast a fully condensed film of oxygen on metallic iron does not oxidise the iron Roberts' experiments on the volatilisation of metals, perhaps, are also a case in point.

During condensation local heat changes occur, the sign being determined by whether the solution of the substance condensed is endothermic or exothermic Let heat be liberated when condensation occurs, the relation of solvent and solute being such that heat is absorbed during solution The local liberation of heat will oppose condensation and the velocity of condensation becomes a function of the rate of dissipation of heat. In the well known case of an over-cooled fluid phase the dissipation of heat may be so slow as completely to arrest the change of phase * If the substance which is being condensed under these conditions is chemically unstable, chemical change of the nature of oxidation, reduction, dissociation or association may be caused locally by the enormous molecular stresses.

This third possibility considers a surface not as specially a place of oxidation because, for instance, oxidation of Würster's tetra-substance occurs there, but as a surface which condenses basic substances In this process oxidation of a basic body may occur, but an equally oxidisable acid substance would escape change

Mr. Drury's experiments clear the ground for further discussion to this extent—they prove conclusively that the condensation of a so-called test substance for "active" oxygen or a simple basic dye not only will take place on to a surface wholly devoid of oxygen, but is actually hindered by the existence thereon of a film of oxygen

It must always be remembered that an oxidation place is also a reduction place, and it is to be called the one or the other according to the particular zero which is chosen. A convenient zero is the chemical potential of atmospheric oxygen, and a place would be an oxidation place if oxygen, whose chemical potential is $\gtreqless$ that of atmospheric oxygen, is condensed to the intramolecular state. Such a region would then be a reduction place for chemical

* H. A. Wilson, 'Camb Phil. Proc.,' vol 10, p 25 (1898)

compounds in which the oxygen potential is ≡ that of atmospheric oxygen, and an oxidation place for substances in which it is less than that of atmospheric oxygen In the absence of some agreement as to the zero point the discussion is likely to be as confused in the future as it has been in the past]

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Studies on Enzyme Action. XXII—*Lipase* (IV)—*The Correlation of Synthetic and Hydrolytic Activity*

By Henry E. Armstrong, F.R.S, and H W Gosney, B Sc

(Received and read April 30, 1914)

In the previous communication on this subject, in which the behaviour of Lipase towards ethereal salts generally was discussed, it has been argued that the enzyme is specially fitted to determine the hydrolysis of the insoluble, oily, glyceric salts of the higher fatty acids but is not suited to act in aqueous solutions. we expressed the opinion that interaction must be supposed to take place at and between surfaces separated only by a thin film of water at most—in other words, that water in excess is inimical to the occurrence of change The results we advanced, in conjunction with those deduced from the study of other enzymes, notably urease, also led us to conclude that it is impossible to apply the laws of mass action directly to the interpretation of the changes effected by Lipase

Previously we have directed our attention only to the hydrolytic activity of the enzyme numerous observations are on record which prove that, whether of animal or vegetable origin, it can act reversibly but no comparative study of the two processes has been made hitherto in the case of fats.*

* (1) Kastle and Löwenhardt, 'Amer Chem Journ.,' vol 24, p 491. (2) Hanriot, 'Compt Rend.,' vol 132, p 212 (1901). (3) Pottevin, *ibid*, vol 136, p. 1152 (1903); (4) 'Bull Soc. Chim,' III, vol 35, p. 693 (1906). (5) Dietz, 'Zeit Physiol Chem.,' vol. 52,

In view of present ignorance of the manner in which fats are formed in the organism and the desirability of determining the extent to which their synthesis can be effected, under various conditions, we have carried out a series of parallel experiments to ascertain the limits within which the two opposing changes take place in presence of different proportions of the interacting substances and of water.

In the first series of synthetic experiments, 4 84 grm of the fatty acids from olive oil (the amount equivalent to 5 grm of the oil) was used, in each case, together with the quantity (0 53 grm) of anhydrous glycerol that would be required if the whole of the fatty acid were to be converted into triglyceride

The acid and glycerol were weighed into a 50-c c. Jena glass flask together with 0 5 grm of the enzyme preparation and 0 5 c c. of toluene The flasks were closed with rubber stoppers and kept slowly rotating in an incubator† maintained at 30° C during the times stated Alcohol was then added and the residual acid titrated with a normal solution of caustic soda. Each determination was made in duplicate and control experiments were carried out simultaneously with a preparation that had been boiled with water to destroy the activity of the enzymes The results are given in the following table —

Table I—Synthesis of Fat from three Molecular Proportions of Acid to one of Glycerol

Time	Acidity of control	Acidity of mixture containing enzyme		Percentage of acid combined
hours				
1	17·00	15·72	15 71	8 0
2	17·07	14 90	15 03	12 4
4	17 06	13·09	12 92	23 9
8	17 04	11 37	11 29	33 6
17	16·93	10·61	10·61	37 9
30	16·91	10 53	10 67	38 0
50	16 65	10 43	10 33	39 2
70	16 70	10 47	10 62	38 3

p. 279 (1907) (6) Hamsik, *ibid.*, vol 59, p 1 (1909) (7) Bradley, 'Journ Biol Chem,' vol. 8, p. 251 (1910) (8) Taylor, 'Univ California Pub Path,' vol. 1, p 33 (1904), (9) 'Journ Biol Chem.,' vol. 2, p. 102 (1906) (10) Fokin, 'Chem Rev Fett-u -Harz. Indust,' vol. 13, p 238 (1906) (11) Welter, 'Zeit. angew Chemie,' vol. 24, p. 385 (1911) (12) Dunlap and Gilbert, 'Amer Chem Soc. Journ,' vol 33, p. 1787 (1911). (13) Kransz, 'Zeit. angew. Chemie,' vol. 24, p 829 (1911). (14) Jalander, 'Biochem. Zeit,' vol. 36, p. 435 (1911). (15) Bournot, *ibid.*, vol. 52, p. 172 (1913)

† That described in the previous communication ('Roy Soc. Proc.,' B, vol. 86, p. 589). It may be noted that the figure there given is printed upside down

To discover whether a true equilibrium had been reached or whether the action had ceased owing to the destruction of the enzyme, 0·5 grm. of enzyme was added to the system after the expiration of 24 hours and the mixture was titrated at the end of a second period of 24 hours. Experiments were also made in which 0 5 and 1 grm of the enzyme were allowed to act during 48 hours before titrating the residual acid.

	Percentage of acid combined
0 5 grm enzyme during 24 hours	37 4
0 5 48	37 7
1·0 48	33 6
0 5 24 Together with 0 5 grm. during a second period of 24 hours	34 9 35 3

The slightly lower activity observed in the experiments with 1 grm of enzyme may have been due to the slight amount of water introduced with the preparation

Further evidence that a true equilibrium had been reached was obtained on hydrolysing olive oil by the theoretical minimum amount of water, *i e* three molecular proportions to each molecular proportion of triglyceride or 5 grm of oil and 0·53 grm of water, quantities equivalent to those used in the synthetic experiments. As in the reverse case, the equilibrium was quickly reached and the acidity of the system was approximately the same as that observed in the experiments in the reverse direction.

Table II —Hydrolysis of Fat by three Molecular Proportions of Water

Time	Percentage of acid liberated
hours	
1	30·6
2	45 5
4	56 0
8	61 3
17	62 0
30	62 9
50	62·6
68	62·0

The addition of even a small amount of water influences the equilibrium to a marked extent and also has a retarding effect—to an increasing extent, moreover, as the amount of water is increased. This is shown in the following table, in which are recorded the results obtained by the synthetic action of 0 5 grm. of enzyme on mixtures of 4 84 grm. of fatty acid from

olive oil and 0·53 grm. of glycerol, together with from 0·31 to 3·1 grm. of water, *i.e.* from 3 to 30 molecules per molecule of glycerol.

Table III.—Synthesis of Fat in Presence of various Molecular Proportions of Water—showing Percentage of Acid combined.*

Time	No water	3 mols.	6 mols.	15 mols.	30 mols.
hours					
1	8·0	7·8	4·7		
2	12·4	12·0	7·3	3·6	
4	23·9	16·3	10·3		2·4
8	33·8	19·4	12·4	5·8	3·5
17	37·9	22·6	13·8	6·0	2·9
30	38·0	22·2		5·9	3·4
50	39·2	22·8		6·7	
70	38·3	21·5	15·2	6·8	3·9

Water also has a marked retarding effect on the rate at which the hydrolysis is effected, as shown in the following table, in which is given the percentage of acid formed on hydrolysing 5 grm. of olive oil in presence of from 3 to 24 molecular proportions of water per molecular proportion of glyceride. In these experiments, the difference between duplicate observations was somewhat greater than in the case of the synthetic experiments.

Table IV.—Hydrolysis of Fat in Presence of various Proportions of Water—showing Percentage of Acid liberated.

Time	3 mols.	6 mols.	9 mols.	15 mols.	24 mols.
hours					
1	30·6	27·2	19·4	13·1	9·6
2	45·5	40·6	27·4	19·1	15·9
4	56·0	61·7	46·7	36·2	23·2
8	61·3	73·1	56·5	49·9	36·7
17	62·0	77·0	74·8	68·0	55·2
30	62·9		83·6	75·7	66·1
50	62·6		85·2	80·2	77·2
70	62·0	78·7	84·8	82·6	81·2

It will be noticed that, in presence of 3, 6 and 9 molecular proportions of water, when equilibrium is reached, the acidity of the system is approximately the same as that observed in the corresponding synthetic series; but that when more water was present the effect on the enzyme was such that the equilibrium was not reached during the experiment.

* The results recorded are in all cases the means of duplicate experiments which differed by about 1 per cent. at most.

The effect of glycerol on the synthetic action is similar to that of water on the hydrolytic change, the equilibrium point being so shifted that more acid is removed from the system. Excess of glycerol retards the rate of change in a very noticeable manner. The amounts of acid which entered into combination in a mixture of 4·82 grm. of fatty acid and 1·06 grm. of glycerol, *i.e.* three molecular proportions of acid to two of glycerol, are shown in the following table

Table V.—Synthesis of Fat in Presence of an excess of one Molecular Proportion of Glycerol.

Time	Acidity of system c.c. normal alkali		Percentage of acid combined
hours			
1	16·01	16·03	6·2
2	15·10	15·18	11·3
4	13·26	13·46	21·8
8	10·45	10·84	37·7
17	8·58	8·51	49·9
30	7·97	8·17	52·7
50	7·56	7·53	55·8
70	7·57	7·55	55·7

When three or more molecular proportions of glycerol are present to every three molecular proportions of acid, the retarding effect is so pronounced that no equilibrium point is reached within a reasonably convenient time, the acidity of the system falling slowly after 70 hours. Thus—

Glycerol, mol. props.	Acidity after 50 hours.	Acidity after 70 hours.
3	45·3	44·5
5	47·3	42·3
10	44·8	40·7

The effect of glycerol on hydrolysis is similar, as is shown in Table VI, in which is recorded the amount of acid liberated from 5 grm. of olive oil by 0·5 grm. enzyme and 0·31 c.c. water, in presence of 0·53 grm. of glycerol.

Table VI.—Hydrolysis of Fat in Presence of one Molecular Proportion of Glycerol

Time	Acidity of system in c.c. normal alkali		Percentage of acid liberated
hours			
2	3 54	3 52	20 6
4	4 98	5·08	29 4
8	6 05	6 13	35 6
17	7·06	7 42	42 3
30	7 73	7 38	44 3
50	7·73		45 3
70	7·70	7·64	44·9

When more glycerol is present hydrolysis takes place to a reduced extent, and still proceeds slowly even after 70 hours.

Glycerol, molecular proportions	Percentage of acid after 50 hours	Percentage of acid after 70 hours
2	29 8	30 9
4	11 0	12 6

The results of the experiments described are summarised in Graphs 1, 2 and 3, the synthetic observations in Graph 1, the hydrolytic in Graph 2, the parallel series of observations in the two opposite directions in Graph 3. The manner in which water affects both the rate of the change and the extent to which this takes place in one or the other direction is brought out in a very striking manner in these diagrams; it will be noticed especially how much less rapid is the approach, both from the hydrolytic and the synthetic side, to an equilibrium as the amount of water present is increased. Whilst the retardation of the hydrolytic change must be ascribed to a direct interference of the water, which presumably prevents the enzyme and the oil from coming into effective contact, the retardation of change in the opposite direction, especially the diminution of the extent to which synthesis takes place, must be ascribed rather to the withdrawal of glycerol from the system through its dissolution in the water; in this connexion, it is remarkable that synthesis is not entirely prevented even by the presence of thirty molecular proportions of water to one of glycerol, whilst in absence of an excess of water, an excess of glycerol beyond two molecular proportions has but little effect in increasing the proportion of fat synthesised.

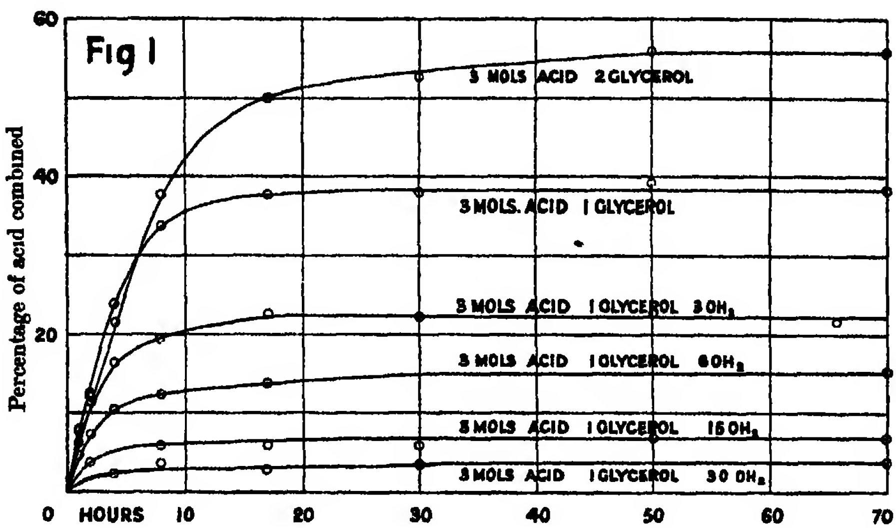
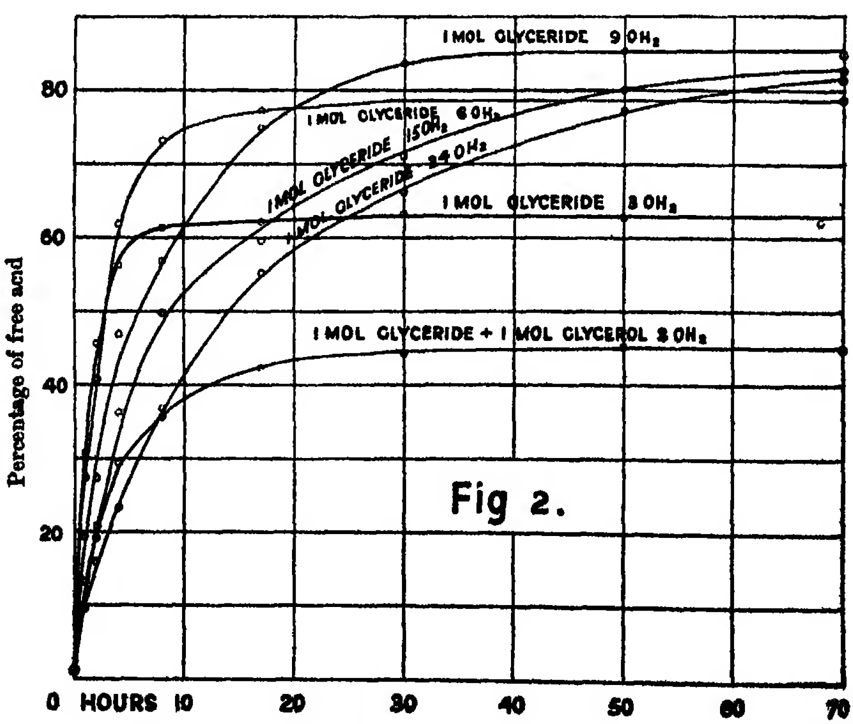
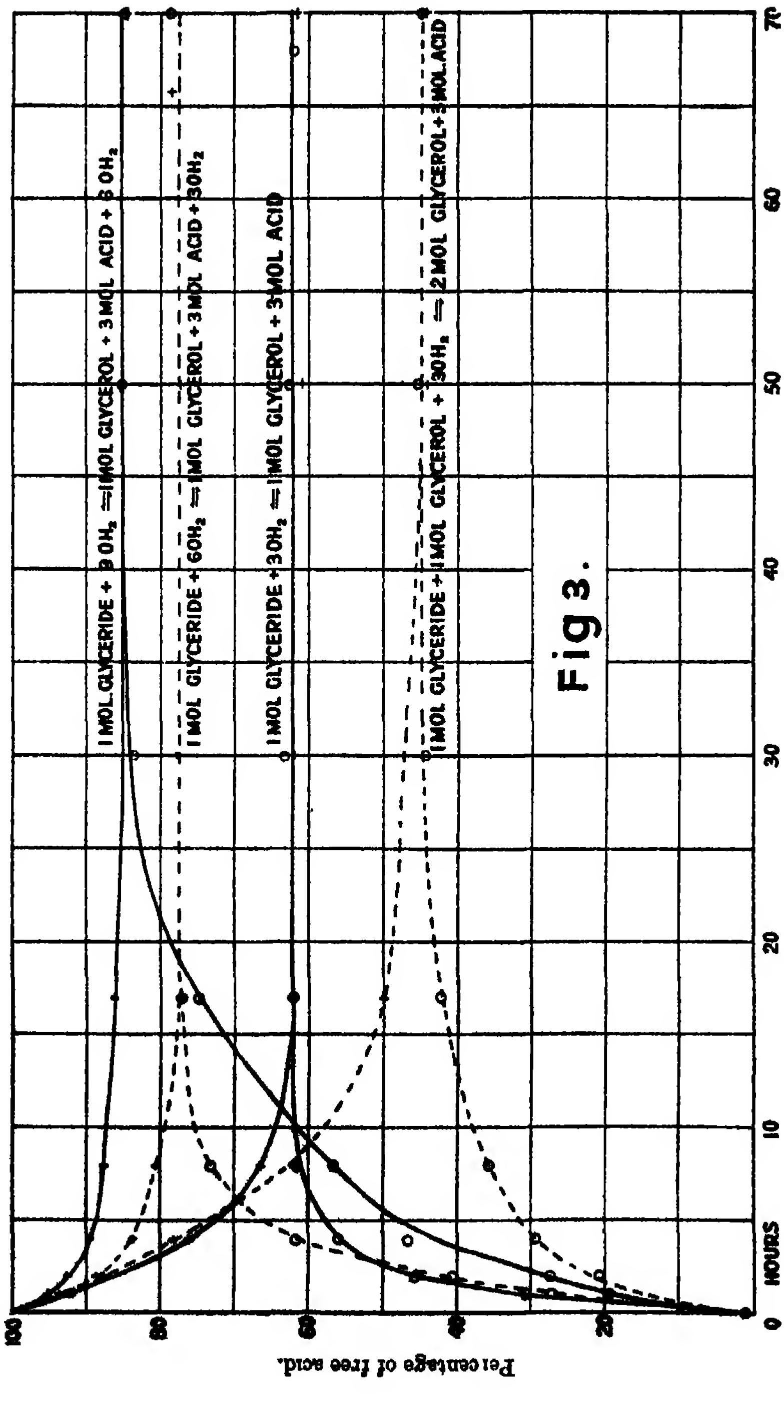


Fig I
3 MOLS ACID 2 GLYCEROL
3 MOLS. ACID 1 GLYCEROL
3 MOLS ACID 1 GLYCEROL 3 OH₂
3 MOLS ACID 1 GLYCEROL 6 OH₂
3 MOLS ACID 1 GLYCEROL 15 OH₂
3 MOLS ACID 1 GLYCEROL 30 OH₂
Percentage of acid combined
60
40
20
0 HOURS 10 20 30 40 50 60 70


1 MOL GLYCERIDE 9 OH₂
1 MOL GLYCERIDE 6 OH₂
1 MOL GLYCERIDE 15 OH₂
1 MOL GLYCERIDE 24 OH₂
1 MOL GLYCERIDE 3 OH₂
1 MOL GLYCERIDE + 1 MOL GLYCEROL 3 OH₂
Fig 2.
Percentage of free acid
80
60
40
20
0 HOURS 10 20 30 40 50 60 70



1 MOL GLYCERIDE + 9 OH$_2$ ⇌ 1 MOL GLYCEROL + 3 MOL ACID + 6 OH$_2$
1 MOL GLYCERIDE + 6OH$_2$ ⇌ 1 MOL GLYCEROL + 3 MOL ACID + 3OH$_2$
1 MOL GLYCERIDE + 3OH$_2$ ⇌ 1 MOL GLYCEROL + 3 MOL ACID
1 MOL GLYCERIDE + 1 MOL GLYCEROL + 3OH$_2$ ⇌ 2 MOL GLYCEROL + 3 MOL ACID
0
HOURS
10
20
30
40
50
60
70
100
80
60
40
20
Percentage of free acid.

Fig 3.

In so far as our results can be brought into comparison with those of previous workers, they appear to be in harmony with their observations. but the activity of the enzyme we have had at our disposal, thanks to Tanaka's important discovery, appears to have been in excess of that used by others.

As it was obvious that if the limit reached in our synthetic experiments (about 40 per cent when equivalents are used) were to be exceeded, the water produced in the interaction must be removed as it is formed, we endeavoured to secure this end by carrying out the synthesis *in vacuo* in a flask connected with drying apparatus The results obtained have been uniformly unsatisfactory, inferior, in fact, to those obtained under ordinary conditions Apparently, as pointed out by us previously, the intervention of a film of water is necessary at the interface of the system, where interaction takes place, if this be removed, action comes to an end

In working with the Tanaka preparation, it is noticeable that the activity varies considerably, in a manner which is difficult to understand at first On more than one occasion we have found that an enzyme which was quite active hydrolytically was inert when used as a synthetic agent with a mixture of acid and glycerol free from water ultimately, this behaviour was traced to "overdrying," as on the addition of a very small amount of water the enzyme became active

Enzyme which has been used and then recovered, by washing it free from oily matter by means of light petroleum, is found, as a rule, to be still active but usually less active than it was originally the variable behaviour of such preparations is not surprising, however, in view of the colloid nature of the material and the effect which alterations in the state of aggregation and of surface conditions must have It is noteworthy that the hydrolytic activity—in presence of a relatively large excess of water—of the enzyme is much more reduced by such treatment than the synthetic activity.

Nature of the Products of Change.—In order to ascertain whether the product of the synthetic action of Lipase is a nearly pure triglyceride like the natural fats and oils, the amount of glycerol uncombined in each experiment of the first series was determined, following the directions given by Lewkowitsch.

After titration, the contents of each flask was washed into an evaporating basin, boiled to expel most of the alcohol and then just acidified by sulphuric acid After heating the liquid to the boiling point, the solution of glycerol was filtered off and the fatty acids and enzyme on the filter were then well washed with hot water: the filtrate was purified by addition of a solution of basic lead acetate and the glycerol estimated in the clear filtrate by Hehner's method (oxidation by an acid solution of potassium bichromate).

The method is probably one which is affected with a considerable error, so that the results have only qualitative significance.

Table VII.

Time	Glycerol found in			Percentage of acid combined	Mols of acid combined per mol glycerol
	Blank series*	Series A	Series B		
hours	grm.	grm	grm		
1	0 51	0 ·08	—	8 1	1 6
2	0 52	0 11	0 11	12 4	1 8
4	0 55	0 18	0 19	23 ·9	2 ·05
8	0 545	0 27	0 28	33 8	2 0
17	0 495	0 28	0 28	37 9	2 15
30	0 535	0 295	0 28	38 0	2 1
50	0 495	0 28	0 305	39 2	2 1
70	0 515	0 ·305	—	38 3	2 ·0

* The amount found should be about 0 53 grm

In the same manner, estimations were made of the amount of glycerol liberated on hydrolysing olive oil by the enzyme in presence of 24 molecular proportions of water It was found that, at first, the acids liberated were slightly in excess of the glycerol, an indication that a small quantity of a lower glyceride was formed but as the action continued, the whole molecule was hydrolysed

Table VIII

Time	Glycerol found		Percentage of acid combined		Mols of acid liberated per mol glycerol
	Series A	Series B	Series A	Series B	
hours	grm				
1	0 ·055	0 045	8 8	8 5	2 8
2	0 ·075	0 ·065	15 ·9	13 8	3 4
8	0 165	0 165	33 2	34 9	3 4
17	0 275	0 25	56 2	53 ·0	3 3
30	0 325	0 33	65 2	64 4	3 1
50	0 ·41	0 ·395	77 5	76 3	3 ·0
70	0 ·43	0 425	80 8	80 9	3 0

The amount of glycerol liberated, however, is less in proportion to the acid when the hydrolysis is brought about by a small proportion of water, showing that under these conditions mono- and di-glycerides are produced to a greater extent

Thus, on hydrolysing 5 grm of oil by 0 31 c.c. of water —

Time	Percentage of acid liberated	Percentage of glycerol liberated	Mols acid per mol glycerol
hours			
1	31·0	24 5	3·8
2	45 6	36 8	3 7
3	61 8	46 3	4·0

Conversely, when the synthesis is effected in presence of water, less glycerol is combined than when no water is added, that is to say, the glycerides formed are more saturated Thus, in presence of 0 62 c c, i e, 6 molecules of water —

Time	Percentage of acid combined	Percentage of glycerol combined	Mols acid per mol glycerol
hours			
2	7 7	12 0	1 9
8	11 9	15·0	2 4
70	15 2	18 5	2 5

An excess of glycerol not only alters the equilibrium so that a greater proportion of acid is combined but also influences the nature of the product, which then contains a smaller proportion of acid · thus the composition of the product of the interaction of two molecular proportions of glycerol and three molecular proportions of acid was found to be as follows —

Table IX

Time	Percentage of acid combined	Percentage of glycerol combined	Mols. acid combined per mol glycerol
hours			
1	6·3	7 1	1·3
2	11 1	9 5	1·8
4	21·2	17 0	1·9
8	38 2	30 2	1·9
17	49 7	41·0	1 8
50	55 7	45 4	1·8

From these results, it is not improbable that the main product is a diglyceride in other words, that, as is to be expected, the two primary hydroxyl groups of glycerol are first affected.

Some of the product of the interaction of the acids from olive oil with an excess of glycerol was isolated by evaporating off the alcohol after neutralising the unchanged acid and extracting the soap solution with ether. About

18 grm. of a pale yellow oil was thus obtained, which became turbid on standing, slowly clearing again on heating to 30° C. The saponification value of this oil was 183.5, that of the olive oil used being 191·7; on acetylation the saponification number was increased to 248·7, the "acetyl value" being 78.6. These data favour the assumption that the oil contained a high proportion of diolein. It is obvious, however, that no final conclusion is possible until experiments have been made with definite acids and the products have been isolated and characterised.

In view of our results, we venture to call attention to several directions in which the fats now deserve renewed attention.

Our knowledge of the manner in which they are absorbed and utilised under vital conditions is at present very vague in character and much of the evidence on which reliance is placed appears to be open to question. It is generally believed that, when ingested, fat is rapidly hydrolysed, under the influence of the pancreatic secretion and that derived from certain tracts of the intestine, this change being regarded as a necessary preliminary to its passage through the walls of the villi prior to entry into the circulatory system. Lipase appears to be widely distributed throughout the organism.

Apparently, whenever fat is to be transferred across cell membranes, it is hydrolysed: assisted by the emulsifying influence of the biliary fluid, the fatty acid that is liberated during digestion of fatty food can penetrate tissues that are impermeable to the fat but it is held that on entry into the villi the fatty acids are rapidly re-associated with glycerol and pass into the lacteals as fat. In fact, all fat that is stored is supposed to be fat that has been reconstituted from fatty acids. In the normal heart and other tissues, however, the fatty acids are not present as glycerides but apparently are combined in such a way that their histological behaviour is different from that of fats—the discriminative staining agents being without effect in such cases.

If the vital mechanism be such that only fatty acids can pass through, it is clear that in presence of lipase fats would undergo complete hydrolysis readily, under natural conditions, if the acids were removed as they were liberated, as reversal would be prevented.

Our observations appear to show that hydrolysis would be most rapid in presence of a minimum amount of water; they therefore favour the conclusion that conditions which would tend to reduce the concentration of the cell fluid would promote the conservation of fat—a conclusion which is perhaps applicable in explanation of the obesity which apparently is a frequent consequence of the indulgence in large quantities of weak alcoholic fluids such as Lager beer.

But in view of our observation that under 40 per cent. of fatty acid is convertible into fat, even when no water is present, it is difficult to understand how the fatty acids are completely reconverted unless there be some mechanism whereby the fat is separated from the fatty acid as it is formed—or some means by which the acid is held in abeyance until it is required. May it not be that the clue is afforded by the observations above referred to with reference to the presence of fat in the tissues in a cryptic form? Lipase apparently is a "carboxylase" which has the power of determining the hydrolysis of the ethereal salts of all the very weak carboxylic acids and, within limits, is more effective the less soluble the acid and the alcohol from which the salt is derived; presumably, the argument applies equally to the synthetic activity of the enzyme. It is therefore probable that, under the influence of lipase, fatty acid may become associated with hydroxylic centres in the protoplasmic complex and that such withdrawal may be the cause of its cryptic existence in muscular tissue.

The effect on health of an absence of fat from the diet, to which Arctic travellers have called attention, is noteworthy from this point of view. Stefánsson, in his recent book 'My Life with the Eskimo,'* states that the symptoms that result from a diet of lean meat are practically those of starvation; during the winter period, even when gorged with caribou meat free from fat, he and his party felt continually hungry; the dogs, though they got more meat than dogs usually get, were nothing but skin and bones. Previously, when they had lived practically on oil alone, taking a teacupful of oil a day, there were no symptoms of hunger; they grew each day sleepier and more slovenly, he says, but at the end of their meal of long-haired caribou skin (to give bulk) and oil felt satisfied and at ease.

On the assumption that fat is not always laid down as such but frequently reconstructed *in situ*, the presence of glycerol in the necessary amount at the seats of synthesis has to be accounted for. Owing to the solubility of this substance, it cannot well be supposed that, when fat is hydrolysed, the fatty acid and glycerol always remain together in the required proportions: it is more probable that the glycerol becomes separated from the acid to a greater or less extent and that the deficit is derived from carbohydrate: it is on this account, at least in part, perhaps, that it is desirable that a certain minimum ratio should be preserved between fat and carbohydrate in our food.

We are indebted to the Hull Oil Manufacturing Company, Ltd., for having placed at our disposal Indian castor seed of recent growth for the purpose of this inquiry.

* Macmillan and Co., London, 1913, pp. 140–141.

[*Note added June* 18.—In a communication which came to our notice only when the work we have described was completed Bournot (15) has called attention to the activity of the lipase present in the seeds of *Chelidonium majus*, the common Celandine, a papaveraceous plant. Having been able, through the courtesy of Messrs. Parke, Davis and Co., to obtain a sample of the seed, we have contrasted its activity with that of our *Ricinus* lipase and have confirmed Bournot's statement that it is not necessary to treat the seed with acid to render it active.

According to Bournot, *Chelidonium* lipase differs from *Ricinus* lipase in being most active in a neutral medium, even N/50 acid having an inhibitory effect. But as is shown in Part II, when once liberated from its zymogen *Ricinus* lipase is also sensitive to acid: in our experience, it has maximum activity when the acidity does not exceed that of oleic acid.

The enzymes from the two sources both hydrolyse and synthesise glyceric oleate with about the same ease and give rise to mixtures similar in composition at the equilibrium point. But weight for weight, the Tanaka *Ricinus* preparation is less active than *Chelidonium* seed (free from oil) in effecting the synthesis of isoprimary butylic oleate. Thus in an experiment in which 41 per cent. of the acid was combined by the agency of the *Ricinus* enzyme, about 80 per cent. was etherified by *Chelidonium* seed. Apparently, the alcohol has a specially marked effect on the *Ricinus* preparation, as olive oil is hydrolysed only to a small extent in presence of a molecular proportion of isobutylic alcohol to one of the oleate.

Similarly, on hydrolysing isobutylic oleate, whereas, in presence of a single molecular proportion of water, 9.3 per cent. of change was effected in 17 hours by the *Chelidonium* enzyme, the *Ricinus* preparation caused only 2.4 per cent. of change. The difference was less marked on using 10 times as much water, as 16.7 per cent. was hydrolysed by the one and 13.0 per cent. by the other "enzyme": in this case, the effect of the alcohol was reduced apparently by the presence of the excess of water.

In our opinion, such differences as are observed are to be regarded, provisionally at all events, as consequences of differences in the "condition" of the enzyme in the different seeds. At present, as it is impossible to arrive at any estimate of the "concentration" of an enzyme or to allow for differences in its distribution, we cannot well make any valid comparison of the enzymes of like function derived from different sources.]

Morphology of Various Strains of the Trypanosome causing Disease in Man in Nyasaland The Human Strain (continued) —VI to X

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INTRODUCTION

In a former paper* five strains of this species of trypanosome, obtained from man, were described In this it is intended to describe another set of five. A small quantity of blood was taken from the sick native, brought up to the laboratory, and inoculated into a monkey or white rat.

Strains VII, VIII, and X were directly inoculated into the rat from which the drawings and measurements were made, in Strains VI and IX a single monkey intervened These five strains have therefore been obtained under fairly similar circumstances, and should prove useful for purposes of comparison This is put in tabular form in Table I, and the history of the previous five strains is also given, as this was omitted in the former paper It is possible that the type of a strain may be changed by passage through different animals before it reaches the rat

Table I —Showing the Passages through Animals between Man and the Rat whose Trypanosomes are Drawn and Measured.

Strain	Man	Monkey	Dog	Rat
I, Mkanyanga	—	—	—	—
II, E——	1	—	2	3
III, Chituluka	1	—	2	3
IV, Chipochola	1	—	2	3
V, Chibibi	1	—	2	3
VI, Manakumpara	1	2	—	3
VII, Yoramu	1	—	—	2
VIII, Mekka	1	—	—	2
IX, Mkanthama	1	2	—	3
X, Dongolosi	1	—	—	2

From this table it will be seen that little time was lost in inoculating the rat, the trypanosomes in whose blood were drawn and measured.

* 'Roy. Soc Proc.,' B, vol 86, p. 285 (1913).

On comparing the curves from the 10 strains it cannot be said that the passage from dog to rat, or from monkey to rat, or direct from man to rat, has had any marked influence on the character of the curve But in these cases only a single monkey or dog intervened

In the case of Strain I, Mkanyanga, the trypanosomes were taken at random from several species of animals,* 600 trypanosomes from four different rats were measured The passages are as follows —(1) Man, monkey, Rat 38, (2) man, dog, rat, rat, Rat 37, (3) man, monkey, guinea-pig, monkey, Rat 236 (4) man, monkey, guinea-pig, monkey, Rat 235

VI Morphology of Strain VI, Manakumpara

The following Table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest —

Table II —Measurements of the Length of the Trypanosome of Strain VI, Manakumpara

Date	Expt No	Animal	Method of fixing	Method of staining	In microns Average length	Maximum length	Minimum length
1913							
June 30	2239	Rat	Osmic acid	Giemsa	21·4	27·0	18·0
,, 30	2239	,,	,,	,,	21·6	29·0	18·0
,, 30	2239	,,	,,	,,	22·1	27·0	20·0
July 1	2239	,,	,,	,,	19·0	23·0	17·0
,, 1	2239	,,	,,	,,	18·6	23·0	16·0
,, 1	2239	,,	,,	,,	18·6	23·0	16·0
,, 2	2239	,,	,,	,,	19·8	22·0	17·0
,, 2	2239	,,	,,	,,	20·6	25·0	17·0
,, 2	2239	,,	,,	,,	19·8	21·0	17·0
,, 3	2239	,,	,,	,,	21·1	27·0	17·0
,, 3	2239	,,	,,	,,	21·8	26·0	17·0
,, 3	2239	,,	,,	,,	22·6	30·0	17·0
,, 4	2239	,,	,,	,,	23·0	26·0	17·0
,, 4	2239	,,	,,	,,	23·0	29·0	18·0
,, 4	2239	,,	,,	,,	23·0	28·0	19·0
,, 5	2239	,,	,,	,,	22·4	28·0	17·0
,, 5	2239	,,	,,	,,	21·8	30·0	17·0
,, 5	2239	,,	,,	,,	23·8	29·0	18·0
,, 7	2239	,,	,,	,,	23·5	28·0	21·0
,, 7	2239	,,	,,	,,	22·9	30·0	17·0
,, 7	2239	,,	,,	,,	24·5	32·0	19·0
,, 8	2239	,,	,,	,,	23·0	28·0	19·0
,, 8	2239	,,	,,	,,	22·6	29·0	17·0
,, 8	2239	,,	,,	,,	21·2	27·0	15·0
,, 9	2239	,,	,,	,,	20·6	26·0	15·0
					21·7	32·0	15·0

* 'Roy Soc. Proc.,' B, vol. 85, p 427 (1912).

Table III.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain VI, Manakumpara, from Rat 2239

	In microns																	
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Totals	3	5	22	40	46	74	82	62	40	37	23	16	23	18	5	3	—	1
Percentages	0·6	1·0	4·4	8·0	9·2	14·8	16·4	12·4	8·0	7·4	4·6	3·2	4·6	3·6	1·0	0·6	—	0·2

Chart 1.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain VI, Manakumpara, taken on nine consecutive days from Rat 2239

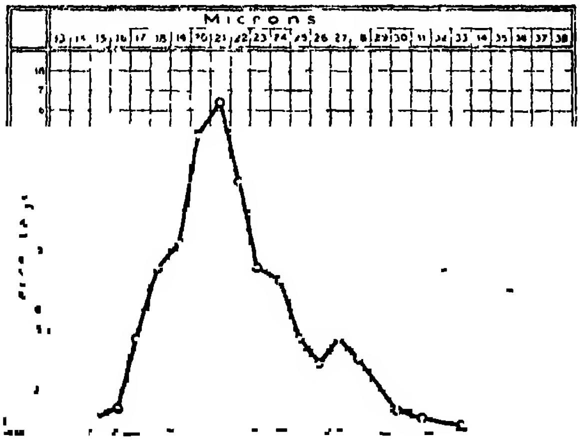


Table IV.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain VI, Manakumpara

Date	Experiment No.	Animal	Percentage among short and stumpy forms
1913			
June 30	2239	Rat	4
July 1	2239	„	16
„ 2	2239	„	16
„ 3	2239	„	14
„ 4	2239	„	9
„ 5	2239	„	21
„ 7	2239	„	8
„ 8	2239	„	18
„ 9	2239	„	17
„ 10	2239	„	25
		Average	14·3

Breadth.—The following Table gives the breadth of the trypanosome of Strain VI, Manakumpara —

Table V—Measurements of the Breadth of the Trypanosome of Strain VI, Manakumpara

Date	Experiment No	Animal	Number of trypanosomes measured	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1913	2239	Rat	500	2 76	4 50	1 25

In regard to shape, contents of cell, size and position of nucleus and micronucleus, disposal of undulating membrane and flagellum, no difference can be made out between the Strains VI to X and the first five strains

VII. Morphology of Strain VII, Yoramu

The following Table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest —

Table VI—Measurements of the Length of the Trypanosome of Strain VII, Yoramu

Date	Expt No	Animal	Method of fixing	Method of staining	In microns		
					Average length	Maximum length	Minimum length
1913							
June 30	2236	Rat	Osmic acid	Giemsa	25 8	28 ·0	23 0
„ 30	2236	„	„	„	26 7	33 0	21 0
„ 30	2236	„	„	„	26 9	31 ·0	22 ·0
July 1	2236	„	„	„	27 0	32 ·0	18 0
„ 1	2236	„	„	„	26 8	32 0	21 0
„ 1	2236	„	„	„	25 8	31 ·0	21 ·0
„ 2	2236	„	„	„	20 3	24 0	18 ·0
„ 2	2236	„	„	„	21 5	26 ·0	18 ·0
„ 2	2236	„	„	„	21 9	29 0	19 0
„ 3	2236	„	„	„	20 5	28 ·0	16 ·0
„ 3	2236	„	„	„	20 2	29 0	16 0
„ 3	2236	„	„	„	19 4	26 0	16 ·0
„ 4	2236	„	„	„	19 4	23 0	17 0
„ 4	2236	„	„	„	19 ·9	24 ·0	18 ·0
„ 4	2236	„	„	„	19 5	22 0	18 ·0
„ 5	2236	„	„	„	22 0	27 ·0	18 ·0
„ 5	2236	„	„	„	21 7	30 ·0	17 0
„ 5	2236	„	„	„	21 ·9	30 0	18 ·0
„ 6	2236	„	„	„	21 7	28 ·0	17 0
„ 6	2236	„	„	„	21 5	28 ·0	17 0
„ 6	2236	„	„	„	22 3	28 ·0	18 0
„ 7	2236	„	„	„	22 5	28 0	18 0
„ 7	2236	„	„	„	22 5	28 0	18 0
„ 7	2236	„	„	„	22 ·0	29 ·0	18 ·0
„ 8	2236	„	„	„	22 4	34 ·0	18 0
					22 5	34 ·0	16 ·0

Table VII —Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain VII, Yoramu, from Rat 2236

	In microns																		
	16	17	18	19	20.	21	22	23	24.	25	26	27	28	29	30	31	32	33	34
Totals	3	9	40	84	67	38	47	27	28	31	33	37	21	13	13	5	2	1	1
Percentages	0 6	1 8	8 0	16 8	13 4	7 6	9 4	5 4	5 6	6·2	6 6	7 4	4 2	2 6	2·6	1 0	0 4	0 2	0·2

Chart 2 —Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain VII, Yoramu, taken on nine consecutive days from Rat 2236

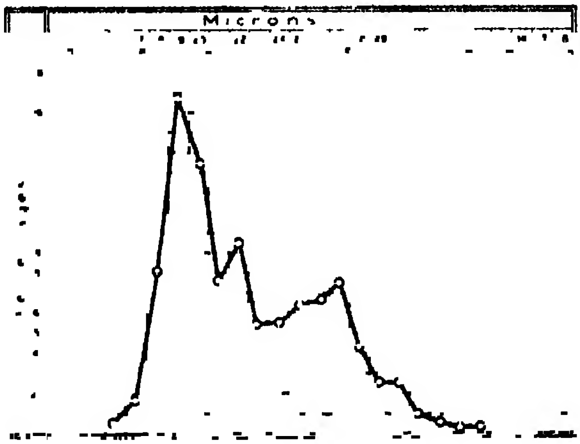


Table VIII —Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain VII, Yoramu

Date	Experiment No	Animal	Percentage among short and stumpy forms
1913			
June 30	2236	Rat	0
July 1	2236	"	1
" 2	2236	"	15
" 3	2236	"	0
" 4	2236	"	2
" 5	2236	"	0
" 7	2236	"	10
" 8	2236	"	36
" 9	2236	"	34
" 10	2236	"	36
	Average		13 4

Breadth—The following Table gives the breadth of the trypanosome of Strain VII, Yoramu:—

Table IX.—Measurements of the Breadth of the Trypanosome of Strain VII, Yoramu

Date	Experiment No	Animal	Number of trypanosomes measured.	In microns		
				Average breadth	Maximum breadth.	Minimum breadth
1913	2236	Rat	500	2·51	4·50	1·25

VIII Morphology of Strain VIII, Mekka

The following Table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table X—Measurements of the Length of the Trypanosome of Strain VIII, Mekka

Date	Expt No	Animal	Method of fixing	Method of staining	In microns		
					Average length	Maximum length	Minimum length
1913							
Aug 27	2300	Rat	Osmic acid	Giemsa	21·2	26·0	19·0
" 27	2300	"	"	"	23·2	29·0	20·0
" 27	2300	"	"	"	21·1	28·0	18·0
" 28	2300	"	"	"	22·2	27·0	19·0
" 28	2300	"	"	"	22·4	26·0	18·0
" 28	2300	"	"	"	23·4	29·0	20·0
" 29	2300	"	"	"	21·4	26·0	20·0
" 29	2300	"	"	"	23·0	28·0	21·0
" 29	2300	"	"	"	21·0	24·0	18·0
" 30	2300	"	"	"	20·4	23·0	18·0
" 30	2300	"	"	"	22·1	28·0	18·0
" 30	2300	"	"	"	20·7	25·0	18·0
" 31	2300	"	"	"	20·7	24·0	18·0
" 31	2300	"	"	"	21·4	29·0	18·0
" 31	2300	"	"	"	21·1	27·0	18·0
Sept. 1	2300	"	"	"	24·8	32·0	20·0
" 1	2300	"	"	"	23·8	33·0	20·0
" 1	2300	"	"	"	24·1	28·0	19·0
" 2	2300	"	"	"	23·9	30·0	19·0
" 2	2300	"	"	"	23·6	29·0	16·0
" 2	2300	"	"	"	23·2	30·0	20·0
" 3	2300	"	"	"	24·0	32·0	20·0
" 3	2300	"	"	"	23·1	29·0	20·0
" 3	2300	"	"	"	23·5	30·0	21·0
" 4	2300	"	"	"	21·3	27·0	18·0
					22·4	33·0	16·0

Table XI.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain VIII, Mekka, from Rat 2300.

	In microns																	
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Totals	1	—	19	30	69	89	80	68	49	34	20	8	10	13	5	2	2	1
Percentages	0·2	—	3·8	6·0	13·8	17·8	16·0	13·6	9·8	6·8	4·0	1·6	2·0	2·6	1·0	0·4	0·4	0·2

CHART 3.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain VIII, Mekka, taken on nine consecutive days from Rat 2300.

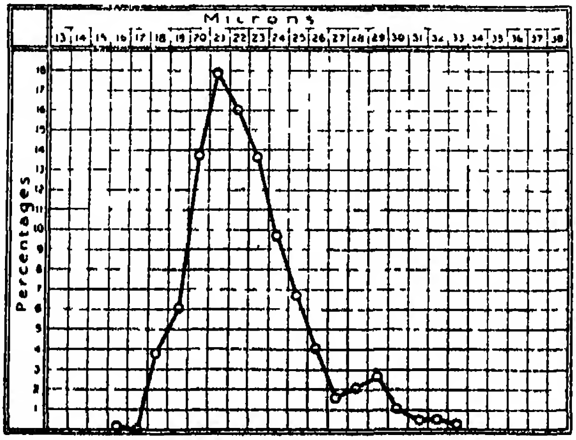


Table XII.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain VIII, Mekka.

Date	Experiment No.	Animal	Percentage among short and stumpy forms
1913.			
July 27	2300	Rat	5
„ 28	2300	„	8
„ 29	2300	„	22
Aug. 2	2300	„	9
„ 3	2300	„	13
„ 7	2300	„	38
„ 8	2300	„	40
„ 9	2300	„	41
„ 11	2300	„	37
„ 14	2300	„	29
		Average	24·2

Breadth—The following Table gives the breadth of the trypanosome of Strain VIII, Mekka —

Table XIII—Measurements of the Breadth of the Trypanosome of Strain VIII, Mekka

Date	Experiment No	Animal	Number of trypanosomes measured	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1913	2300	Rat	500	2·68	5·00	1·25

IX Morphology of Strain IX, Mkanthama

The following Table gives the average length of this trypanosome as found in the white rat, 500 trypanosomes in all, and also the longest and shortest —

Table XIV—Measurements of the Length of the Trypanosome of Strain IX, Mkanthama

Date	Expt No	Animal	Method of fixing	Method of staining	In microns		
					Average length	Maximum length	Minimum length
1913							
Aug 18	2386	Rat	Osmic acid	Giemsa	23·2	31·0	17·0
„ 18	2386	„	„	„	26·7	33·0	18·0
„ 18	2386	„	„	„	25·1	32·0	18·0
„ 19	2386	„	„	„	19·0	24·0	17·0
„ 19	2386	„	„	„	18·5	26·0	16·0
„ 19	2386	„	„	„	18·2	23·0	16·0
„ 20	2386	„	„	„	19·4	24·0	17·0
„ 20	2386	„	„	„	19·8	30·0	16·0
„ 20	2386	„	„	„	19·9	30·0	16·0
„ 21	2386	„	„	„	17·7	24·0	15·0
„ 21	2386	„	„	„	17·8	22·0	14·0
„ 21	2386	„	„	„	17·5	20·0	16·0
„ 22	2386	„	„	„	19·4	31·0	15·0
„ 22	2386	„	„	„	20·5	28·0	15·0
„ 22	2386	„	„	„	21·8	29·0	15·0
„ 23	2386	„	„	„	23·5	29·0	17·0
„ 23	2386	„	„	„	24·2	32·0	16·0
„ 23	2386	„	„	„	23·4	31·0	16·0
„ 24	2386	„	„	„	24·1	33·0	18·0
„ 24	2386	„	„	„	23·4	31·0	18·0
„ 24	2386	„	„	„	21·6	30·0	17·0
„ 25	2386	„	„	„	22·0	29·0	17·0
„ 25	2386	„	„	„	21·8	31·0	17·0
„ 25	2386	„	„	„	21·2	30·0	15·0
„ 26	2386	„	„	„	19·6	26·0	17·0
					21·2	33·0	14·0

Table XV.—Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain IX, Mkanthama.

	In microns																			
	14.	15	16	17	18	19.	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Totals	1	9	22	64	79	70	40	23	21	29	23	24	23	16	13	15	12	9	5	2
Percentages	0·2	1·8	4·4	12·8	15·8	14·0	8·0	4·6	4·2	5·8	4·6	4·8	4·6	3·2	2·6	3·0	2·4	1·8	1·0	0·4

CHART 4.—Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain IX, Mkanthama, taken on nine consecutive days from Rat 2386.

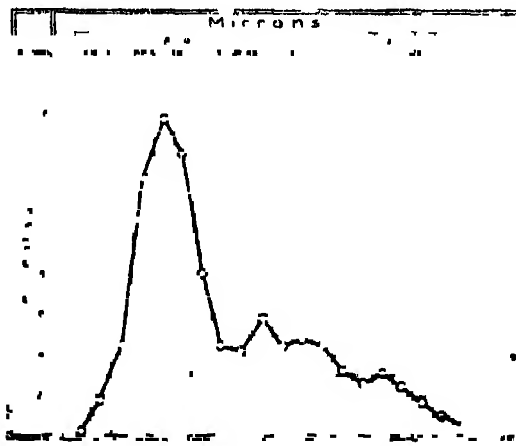


Table XVI.—Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain IX, Mkanthama.

Date	Experiment No.	Animal	Percentage among short and stumpy forms
1913.			
May 18	2386	Rat	13
„ 19	2386	„	32
„ 20	2386	„	18
„ 21	2386	„	11
„ 22	2386	„	19
„ 23	2386	„	27
„ 24	2386	„	34
„ 25	2386	„	33
„ 26	2386	„	45
„ 28	2386	„	54
		Average	28·6

Breadth.—The following Table gives the breadth of the trypanosome of Strain IX, Mkanthama.

Table XVII.—Measurements of the Breadth of the Trypanosome of Strain IX, Mkanthama.

Date	Experiment No.	Animal	Number of trypanosomes measured	In microns. Average breadth	Maximum breadth	Minimum breadth
1913	2386	Rat	500	2·56	5·00	1·25

X. Morphology of Strain X, Dongolosi.

The following Table gives the average length of this trypanosome, as found in the white rat, 500 trypanosomes in all, and also the longest and shortest:—

Table XVIII.—Measurements of the Length of the Trypanosome of Strain X, Dongolosi.

Date	Expt. No.	Animal	Method of fixing	Method of staining	In microns. Average length	Maximum length	Minimum length
1913							
Nov. 27	2437	Rat	Osmic acid	Giemsa	24·8	30·0	20·0
" 27	2437	"	"	"	24·6	29·0	21·0
" 27	2437	"	"	"	25·0	29·0	21·0
" 28	2437	"	"	"	19·9	22·0	17·0
" 28	2437	"	"	"	19·9	23·0	18·0
" 28	2437	"	"	"	19·6	22·0	18·0
Dec. 2	2437	"	"	"	23·2	28·0	17·0
" 2	2437	"	"	"	22·0	29·0	18·0
" 2	2437	"	"	"	23·2	28·0	18·0
" 3	2437	"	"	"	24·0	30·0	20·0
" 3	2437	"	"	"	23·7	29·0	20·0
" 3	2437	"	"	"	24·0	30·0	19·0
" 4	2437	"	"	"	23·2	27·0	19·0
" 4	2437	"	"	"	23·2	30·0	19·0
" 4	2437	"	"	"	23·7	26·0	21·0
" 5	2437	"	"	"	24·3	28·0	20·0
" 5	2437	"	"	"	23·9	29·0	19·0
" 5	2437	"	"	"	24·4	28·0	21·0
" 6	2437	"	"	"	24·9	30·0	20·0
" 6	2437	"	"	"	25·2	30·0	22·0
" 6	2437	"	"	"	24·5	28·0	21·0
" 7	2437	"	"	"	25·3	31·0	20·0
" 7	2437	"	"	"	25·0	30·0	20·0
" 7	2437	"	"	"	24·8	32·0	20·0
" 8	2437	"	"	"	22·3	25·0	18·0
					23·5	32·0	17·0

Table XIX — Distribution in respect to Length of 500 Individuals of the Trypanosome of Strain X, Dongolosi

	In microns															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Totals	2	9	30	36	51	52	69	68	61	48	25	23	11	12	2	1
Percentages	0·4	1·8	6·0	7·2	10·2	10·4	13·8	13·6	12·2	9·6	5·0	4·6	2·2	2·4	0·4	0·2

Chart 5 — Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X, Dongolosi, taken on nine consecutive days from Rat 2437, at the beginning of the infection

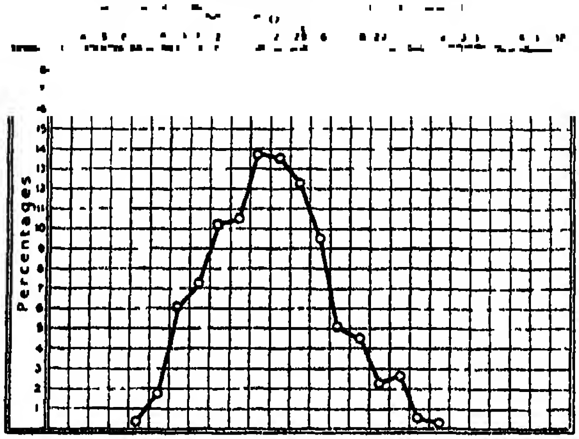
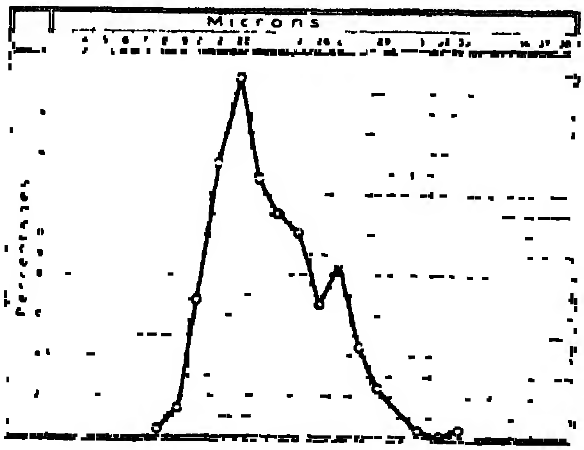


Chart 6 — Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X, Dongolosi, taken on the 33rd to 41st day of disease from Rat 2462



It is evident that in this case there is little difference in type in the trypanosomes taken during the first nine days and the last nine days of an infection In the former there are 25 per cent short and stumpy forms, 37 per cent intermediate, and 38 per cent long and slender, in the latter 22, 43, and 35 per cent respectively

Table XX — Percentage of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Trypanosome of Strain X, Dongolosi

Date	Experiment No	Animal	Percentage among short and stumpy forms
1913			
Nov 27	2437	Rat	1
„ 28	2437	„	3
Dec 2	2437	„	0
„ 3	2437	„	1
„ 4	2437	„	10
„ 5	2437	„	8
„ 6	2437	„	4
„ 7	2437	„	7
„ 8	2437	„	10
„ 9	2437	„	11
		Average	5 0

Breadth —The following Table gives the breadth of the trypanosome of Strain X, Dongolosi

Table XXI —Measurements of the Breadth of the Trypanosome of Strain X, Dongolosi

Date	Experiment No	Animal	Number of trypanosomes measured	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1913	2437	Rat	500	2 71	4 50	1 25

To Ascertain the Type of Trypanosome which arises from a Single Trypanosome.

There is always, when dealing with a dimorphic type of trypanosome, a danger of there being two species present. Some experiments were, therefore, made by inoculating animals with a single trypanosome, to find out if the original dimorphic type would appear The single trypanosomes were picked out in the usual way, by means of dilution and capillary tubes The blood of

an infected rat was diluted with normal saline solution until a volume one-sixteenth of an inch in length of a fine capillary tube was found to contain a single trypanosome This was then inoculated into a rat. Six experiments were made, three of which were successful In each case the long and slender type of trypanosome was isolated and injected

As will be seen from the following curves, from the single long and slender type, short and stumpy, intermediate, and long and slender forms resulted Among the short and stumpy there was a large percentage of the blunt-ended posterior-nucleated forms, which are a feature of this species of trypanosome

From these three experiments, then, it may be concluded that in the dimorphic trypanosome causing disease in man in Nyasaland, a single species is being dealt with

CHART 7 —Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X, Dongolosi, taken on nine consecutive days from Rat 2493, which had been infected by a single trypanosome of the long and slender type

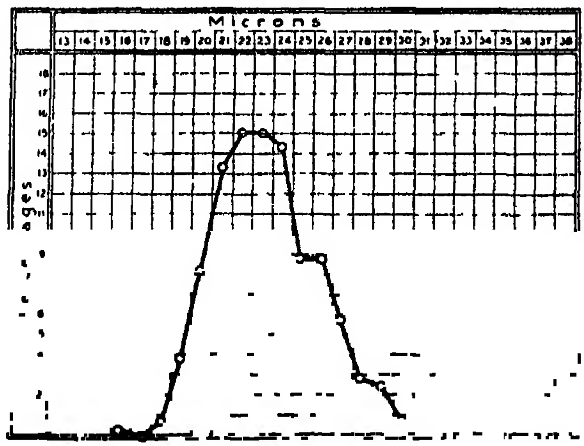
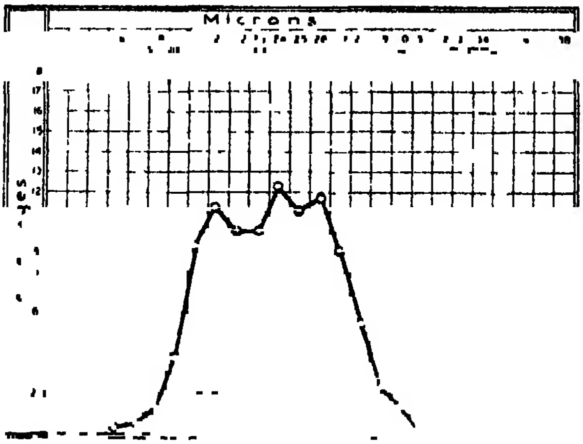


CHART 8 —Curve representing the Distribution, by Percentages, in respect to Length, of 500 Individuals of the Trypanosome of Strain X, Dongolosi, taken on nine consecutive days from Rat 2489, which had been infected by a single trypanosome of the long and slender type



COMPARISON OF THE HUMAN STRAINS VI TO X

Table XXII —Measurements of the Length of the Trypanosomes of the Five Human Strains VI to X The trypanosomes have been taken from the rat alone

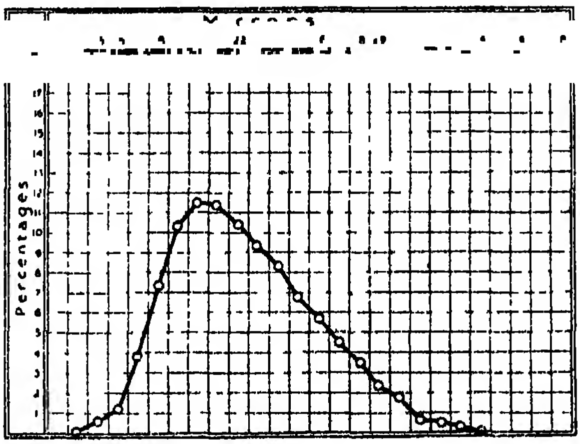
Date	Strain	Name	Number of trypanosomes measured	Average length	In microns: Maximum length	Minimum length
1913	VI	Manakumpara	500	21·7	32·0	15·0
1913	VII	Yoramu	500	22·5	34·0	16·0
1913	VIII	Mekka	500	22·4	33·0	16·0
1913	IX	Mkanthama	500	21·2	33·0	14·0
	X	Dongolosi	500	23·5	32·0	17·0
				22·3	34·0	14·0

The average length of Strains I to V, taken from rats alone, is 24·2 microns, maximum 38, minimum 15 This gives an average for the 10 strains of 23·2 microns, maximum 38, minimum 14

Table XXIII.—Distribution in respect to Length of 2500 Individuals of the Human Strains VI to X. The trypanosomes have been taken from the rat alone.

	In microns																				
	14.	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Totals	1	12	31	97	187	260	286	283	262	233	205	173	140	109	85	57	45	18	11	4	1
Percentages	0·05	0·5	1·2	3·9	7·4	10·4	11·4	11·3	10·4	9·3	8·2	6·9	5·6	4·4	3·4	2·3	1·8	0·7	0·4	0·2	0·05

CHART 9.—Curve representing the Distribution, by Percentages, in respect to Length, of 2500 Individuals of the Human Strains VI to X of the Trypanosome causing Disease in Man in Nyasaland, taken from the Rat alone.



This curve is very similar to that made from Strains I to V,* except that it lies a little to the shorter side.

Table XXIV.—Comparison of the Percentages of Posterior-nuclear Forms found among the Short and Stumpy Varieties of the Human Strains VI to X.

Date	Experiment No.	Strain	Animal	Percentage among short and stumpy forms
1913	2239	VI, Manakumpara	Rat	14·3
1913	2236	VII, Yoramu	"	13·4
1913	2300	VIII, Mekka	"	24·2
1913	2346	IX, Mkanthama	"	28·6
1913	2437	X, Dongolosi	"	5·0
			Average	17·1

The average percentage of the Strains I to V was 17·8 microns.

* 'Roy. Soc. Proc.,' B, vol. 86, p. 301 (1913).

Table XXV.—Comparison of the Measurements of the Breadth of the Trypanosomes of the Human Strains VI to X.

Date	Experiment No.	Strain	Animal	In microns		
				Average breadth	Maximum breadth	Minimum breadth
1913	2239	VI, Manakumpara	Rat	2·76	4·50	1·25
1913	2236	VII, Yoramu	„	2·51	4·50	1·25
1913	2300	VIII, Mekka	„	2·68	5·00	1·25
1913	2386	IX, Mkanthama	„	2·56	5·00	1·25
1913	2437	X, Dongolosi	„	2·71	4·50	1·25
				2·65	5·00	1·25

Conclusion

These further five strains of this trypanosome, isolated from five natives in Nyasaland, belong to the same species, *Trypanosoma brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland.

The Trypanosome causing Disease in Man in Nyasaland. II. *The Wild-game Strain.* III. *The Wild* Glossina morsitans *Strain.* Part II.—*Susceptibility of Animals.*

By Surgeon-General Sir David Bruce, C.B., F.R.S., A.M.S., Major A. E. Hamerton, D.S.O., and Captain D. P. Watson, R.A.M.C., and Lady Bruce, R.R.C. (Scientific Commission of the Royal Society, Nyasaland, 1912–14.)

(Received May 5,—Read June 25, 1914.)

Introduction

In previous papers* the morphology of these strains of trypanosomes was described, and it was concluded that they are identical with the trypanosome causing disease in man in Nyasaland, the *Trypanosoma rhodesiense* of Stephens and Fantham, the *T. brucei* of this Commission.

This paper tabulates the action on animals of the two strains, and they are compared in this respect with each other and with the Human strain.

* 'Roy. Soc. Proc.,' B, vol. 86, pp. 394 and 408.

Animals Susceptible to the Trypanosome causing Disease in Man in Nyasaland

II *The Wild-game Strain*

Table I

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks
			Goat.		
1912					
July 1	718	Hartebeeste 779	7	67	Died of Wild game strain
" 23	796	" 957	13	60	" "
" 27	975	" 1000	9	27	" "
Aug 21	1126	" 1142	5	25	" "
" 24	1121	Eland 1202	16	82	Also showed *T pecorum*
" 24	1127	Waterbuck 1180	9	49	Died of Wild game strain
" 28	1213	" 1210	10	49	Also showed *T capræ*
		Average	8 6	45 6	
			Monkey		
June 19	785	Reedbuck 783	8	17	Died of Wild game strain
July 1	776	Hartebeeste 779	7	30	" "
" 8	864	Oribi 868	7	59	" "
" 23	961	Hartebeeste 957	13	21	" "
" 27	1001	" 1000	9	10	" "
Aug 24	1181	Waterbuck 1180	9	40	" "
" 28	1211	" 1210	8	79	" "
Sept 13	1348	Reedbuck 1347	10	19	" "
" 23	1436	" 1435	10	66	" "
		Average	9·0	39 9	
			Dog		
June 30	784	Reedbuck 783	8	11	Died of Wild game strain
July 1	733	Hartebeeste 779	7	58	" "
" 10	846	Monkey 785	5	48	" "
" 10	848	Dog 733	8	43	" "
" 23	892	Hartebeeste 957	6	31	" "
" 26	991	Monkey 864	6	48	" "
" 27	1002	Hartebeeste 1000	9	59	" "
Aug 21	1144	" 1142	1	29	" "
" 24	1182	Waterbuck 1180	9	40	" "
" 28	1212	" 1210	8	26	" "
Sept 7	1266	" 1264	16	64	" "
" 18	1349	Reedbuck 1347	10	38	" "
" 23	1437	" 1435	10	42	" "
		Average	7 9	41·2	
			Rat.		
July 10	847	Monkey 785	5	30	Died of Wild-game strain
" 10	849	Dog 733	8	39	" "
" 26	992	Monkey 864	6	21	" "
Aug 13	1070	Dog 1002	6	17	" "
" 16	1022	" 892	3	30	" "
Sept 3	1220	Monkey 1181	3	54	" "
		Average	5 2	31 8	

* Duration includes the days of incubation; it dates from the day of inoculation.

In making up the average incubation and duration, mixed infections are not included. It must be admitted that these averages are only approximate, as it is impossible to deal only with animals of the same age, weight, health, and powers of resistance. Dogs, for example, fall away very much during the rains, when biting flies and ticks are numerous.

Table II.—The Average Duration, in Days, of the Disease in Various Animals caused by the Wild-game Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Goat	Monkey	Dog	White rat
Average duration, in days	46	38	41	32
No. of animals employed	5	9	13	6

Compare this with the following Table:—

Table III.—The Average Duration of Life, in Days, of Various Animals infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Goat	Monkey	Dog	White rat
Average duration, in days	42	28	24	30
No. of animals employed	29	20	25	21

Table IV.—The Percentages of Recoveries in Various Animals infected with the Wild-game Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Goat	Monkey	Dog	White rat
Percentages ..	0	0	0	0
No. of animals employed	5	9	13	6

Compare this with the following Table:—

Table V.—The Percentages of Recoveries in Various Animals infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Goat.	Monkey.	Dog.	White rat.
Percentages	0	0	0	0
No. of animals employed	20	20	25	21

III. *The Wild* Glossina morsitans *Strain*.

Table VI.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1912					
April 18	437	Dog 325	9	—	Recovered.
" 18	438	" 325	9	—	"
			Goat.		
Jan. 21	35	Wild flies	19	85	Died of wild fly strain.
Feb. 1	117	Monkey 20	11	50	Also showed *T. simiæ*.
" 13	202	Wild flies	6	24	Died of wild fly strain.
" 17	201	Dog 116	12	27	" "
" 17	207	" 116	9	40	" "
April 13	421	" 325	19	130	" "
" 13	423	" 325	9	82	" "
" 13	424	" 325	9	93	" "
May 15	416	Wild flies	5	19	Also showed *T. simiæ* and *T. pecorum*.
June 12	637	Rat 548	29	82	Died of wild fly strain.
" 12	638	" 548	12	46	" "
" 12	639	" 548	12	—	Still alive after 224 days.
" 27	716	Wild flies	10	60	Died of wild fly strain.
Oct. 31	1538	"	8	24	Also showed *T. pecorum* and *T. capræ*.
Nov. 23	1626	"	7	56	Died of wild fly strain.
" 27	1638	"	15	64	Also showed *T. pecorum*.
Dec. 5	1667	"	11	80	" *T. pecorum* and *T. capræ*.
" 9	1676	"	4	40	" *T. capræ*.
" 13	1685	"	7	38	" *T. pecorum*.
1913					
April 16	2084	Rat 2020	5	41	Died of wild fly strain.
" 16	2085	" 2020	19	32	" "
" 16	2086	" 2020	8	32	" "
" 16	2087	" 2020	8	39	" "
" 16	2088	" 2020	8	44	" "
		Average	11.8	54.3	
			Pig.		
1912					
Nov. 25	1636	Wild flies	14	98	Also showed *T. simiæ* and *T. pecorum*.
1913					
Jan. 21	1781	"	6	230	" *T. pecorum*.
April 12	2075	"	11	24	" *T. simiæ*.
May 14	2169	"	8	25	" "

* Duration includes the days of incubation; it dates from day of inoculation.

Table VI—*continued*

Date.	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks.
			Monkey		
1912					
Feb 26	287	Dog 157	7	28	Died of wild fly strain
„ 27	286	„ 211	6	52	Also showed *T simiæ*
Mar 13	333	Goat 117	8	31	Died of wild fly strain
April 13	406	Dog 325	9	71	„ „
„ 27	492	„ 436	5	63	„ „
May 8	523	Wild flies	4	5	Also showed *T simiæ*
„ 31	601	„	6	31	„ *T pecorum*
June 7	625	„	9	83	Died of wild fly strain
„ 25	739	„	5	45	„ „
July 24	970	Rat 658	8	—	Still alive after 162 days
Sept 27	1459	Wild flies	9	13	Died of wild fly strain
Oct 29	1536	„	10	12	„ „
1913					
Jan 13	1757	„	6	32	„
May 14	2151	Rat 2082	5	30	„ „
„ 14	2152	„ 2082	5	41	„ „
„ 14	2153	„ 2082	5	22	„ „
„ 14	2154	„ 2082	5	28	„ „
„ 14	2155	„ 2082	8	33	„ „
		Average	6·9	38 7	
			Dog		
1912					
Feb 1	116	Monkey 20	8	28	Died of wild fly strain
„ 16	211	Wild flies	6	—	Killed March 2
„ 17	157	Dog 116	9	11	Died of wild fly strain
„ 17	243	„ 116	5	23	„ „
„ 17	244	„ 116	5	23	„ „
Mar 9	325	Monkey 286	9	41	„ „
April 12	436	Wild flies	7	36	„ „
„ 13	440	Dog 325	5	29	„ „
„ 13	441	„ 325	9	60	„ „
„ 27	493	„ 436	5	60	„ „
May 10	535	Wild flies	3	8	„ „
„ 13	542	„	6	42	„ „
„ 17	549	Monkey 523	10	51	Also showed *T pecorum*
„ 17	551	Wild flies	8	25	„ „
„ 29	595	„	3	18	„ „
„ 31	602	„	5	—	Still alive after 175 days
June 8	626	„	8	58	Died of wild fly strain
„ 26	729	„	5	26	„ „
July 24	971	Rat 658	8	32	„ „
Oct. 30	1537	Wild flies	5	30	„ „
Nov 22	1635	„	4	30	„ „
„ 26	1637	„	12	38	„ „
Dec. 7	1675	„	6	25	„ „
„ 12	1684	„	4	19	„ „
1913.					
Jan. 22	1782	Wild flies	9	21	„ „
May 14	2146	Rat 2082	5	17	„ „
„ 14	2147	„ 2082	8	17	„ „
„ 14	2148	„ 2082	8	17	„ „
„ 14	2149	„ 2082	5	11	„ „
„ 14	2150	„ 2082	5	24	„ „
		Average	6·4	28·6	

* Duration includes the days of incubation; it dates from day of inoculation

Table VI—*continued.*

Date	No of expt	Source of virus	Period of incubation, in days	Duration of disease, in days *	Remarks.
			Rabbit		
1912.					
April 13	439	Dog 325	—	—	Never showed trypanosomes
Dec 14	1543	Pig 1636	19	13	Died of wild fly strain
" 14	1544	" 1636	19	39	" "
" 14	1545	" 1636	33	90	" "
		Average	23 7	47 3	
			Guinea-pig		
Feb 17	239	Dog 116	16	80	Died of wild fly strain
" 17	240	" 116	16	72	" "
April 13	442	" 325	—	—	Never showed trypanosomes.
May 14	544	Monkey 492	—	—	" "
June 14	676	Dog 549	—	—	" "
" 14	677	" 549	—	—	" "
" 14	678	" 542	13	116	Died of wild fly strain
" 14	679	" 542	20	104	" "
" 14	680	" 551	13	39	Also showed *T pecorum*
" 14	681	" 551	20	42	" "
" 14	682	" 595	—	—	Never showed trypanosomes
" 14	683	" 595	—	—	" "
1913					
Jan 4	1731	Wild flies	15	100	Died of wild fly strain
Mar 28	2034	Guinea pig 1731	10	53	" "
" 28	2035	" 1731	10	89	" "
April 16	2077	Rat 2020	15	61	" "
" 16	2078	" 2020	12	61	" "
" 16	2079	" 2020	8	72	" "
		Average	13 5	80 8	
			Rat.		
1912					
Feb 17	241	Dog 116	5	17	Died of wild fly strain
" 17	242	" 116	5	15	" "
April 13	443	" 325	9	24	" "
" 27	494	" 436	12	14	" "
May 7	519	" 440	6	12	" "
" 14	548	Monkey 492	6	29	" "
" 17	550	Rat 519	6	12	" "
June 11	655	Dog 549	5	36	" "
" 11	656	" 542	6	19	" "
" 11	657	" 551	13	31	Also showed *T pecorum*
" 11	658	" 595	8	48	Died of wild fly strain
" 12	660	" 602	5	70	Also showed *T pecorum*
Dec 3	1664	Monkey 970	13	40	Died of wild fly strain.
1913					
Jan 13	1755	Rat 1664	7	71	" "
Mar 25	2020	" 1755	13	22	" "
" 25	2021	" 1755	13	22	" "
April 16	2080	" 2020	1	27	" "
" 16	2081	" 2020	5	31	" "
" 16	2082	" 2020	5	28	" "
" 16	2083	" 2020	5	27	" "
May 15	2166	" 2082	8	11	" "
		Average	7 3	26·3	

* Duration includes the days of incubation; it dates from the day of inoculation.

Table VII.—The Average Duration, in Days, of the Disease in Various Animals caused by the Wild *Glossina morsitans* Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Average duration, in days	Rec	54	38	29	47	81	26
No. of animals employed	2	16	14	25	3	10	19

Compare this with the following Table:—

Table VIII.—The Average Duration of Life, in Days, of Various Animals infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Average duration, in days	134	42	26	34	28	67	30
No. of animals employed	1	29	20	25	7	15	21

Table IX.—The Percentages of Recoveries in Various Animals infected with the Wild *Glossina morsitans* Strain of the Trypanosome causing Disease in Man in Nyasaland.

	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Percentages	100	6	7	4	0	0	0
No. of animals employed	2	17	15	26	3	10	19

Compare this with the following Table:—

Table X—The Percentages of Recoveries in Various Animals infected with the Human Strain of the Trypanosome causing Disease in Man in Nyasaland

	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Percentages	80	0	0	0	0	0	0
No of animals employed	5	20	20	25	7	15	21

COMPARISON OF THE WILD-GAME AND WILD GLOSSINA MORSITANS STRAINS WITH THE HUMAN STRAIN OF THE TRYPANOSOME CAUSING DISEASE IN MAN IN NYASALAND

Table XI—The Average Duration, in Days, of the Wild-game, Wild *Glossina morsitans* and Human Strains of the Trypanosome causing Disease in Man in Nyasaland, in regard to their Virulence towards Various Animals

Strain	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Human	134	42	26	34	28	67	30
Wild game	—	40	38	41	—	—	32
Wild *G morsitans*	Rec	54	38	29	47	81	26

Table XII—The Percentages of Recoveries in Various Animals of the Wild-game, Wild *Glossina morsitans* and Human Strains of the Trypanosome causing Disease in Man in Nyasaland

Strain	Ox	Goat	Monkey	Dog	Rabbit	Guinea-pig	White rat
Human	80	0	0	0	0	0	0
Wild game	--	0	0	0	—	—	0
Wild *G morsitans*	100	6	7	4	0	0	0

CONCLUSIONS

1 The pathogenic action on various animals of the Human strain, the Wild-game strain and the Wild *G morsitans* strain is so much alike, that it may be concluded that they all three belong to the same species of trypanosome

2 This species is *T brucei vel rhodesiense*, the trypanosome causing disease man in Nyasaland

The Trypanosome causing Disease in Man in Nyasaland The Naturally Infected Dog Strain. Part III—*Development in* Glossina morsitans

By Surgeon-General Sir DAVID BRUCE, C B, F R S, A M S, Major A E HAMERTON, D S O, and Captain D P WATSON, R A M C., and Lady BRUCE, R R C (Scientific Commission of the Royal Society, Nyasaland, 1912–14)

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INTRODUCTION.

In previous papers* the morphology and action on animals of this strain of trypanosome were described In this a short account of its development in *Glossina morsitans* is given, in order to compare it with the development of the Human strain of the trypanosome causing disease in man in Nyasaland †

It is to be regretted that more material is not available, but, scanty as it is, there is enough to show that this strain develops in the alimentary tract and salivary glands of *G morsitans* in the same way as the *Trypanosoma brucei* and *gambiense* group The Commission aimed at having five positive experiments in every series of transmission experiments, but in this case failed The failure was principally due to the difficulty of procuring laboratory-bred flies, and also to the fact that this strain of trypanosome does not readily develop in *G morsitans*

THE DEVELOPMENT OF THE NATURALLY INFECTED DOG STRAIN IN G MORSITANS

Eleven experiments were made with laboratory-bred flies Two were positive and nine negative

Three hundred and seventy-six flies were used and fourteen were found infected—3 7 per cent This small percentage is partly due to the fact that in some of the experiments few or none of the flies were dissected. There is the same discrepancy to be noted here as in *T brucei*, Zululand, 1913 In some of the experiments not a single infected fly was found, whereas in Experiment 2018A there were seven in a cageful of 36.

* 'Roy Soc. Proc,' B, vol 88, pp 111 and 130 (1914)

† *Ibid* B, vol. 87, p 515

Table I.—Laboratory-bred Flies

Date	Expt.	No. of flies used	Experiment positive or negative	No. of flies dissected	No. of infected flies found	No. of days before flies became infective	Temperature at which flies kept
1912							
Sept. 7	1257	25	–	25	0		84° F. (29° C.)
Oct. 23	1499	26	–	26	1		,,
Dec. 3	1668	10	–	10	0		,,
1913							
Jan. 13	1753	50	–	18	0		84° F. (29° C.)
Mar. 24	2018	55	–	18	2		,,
April 7	2067	30	+	30	3	24	,,
May 9	2018A	40	–	36	7		,,
June 13	2226	40	–	8	1		,,
July 21	2303	40	–	29	0		,,
Aug. 30	2394	40	+	0	0	58	,,
Nov. 12	2483	20	–	15	0		,,

Details of the Two Positive Experiments.

The following Table gives the details in the carrying out of the two positive experiments. They were both carried out with laboratory-bred flies.

Table II.

Date	Day of expt.	Procedure	Result: Positive	Result: Negative	Remarks
		Experiment 2067.			
1913					
April 7	—	30 flies fed on infected Rat 2024			
,, 8	1				
,, 9	2				
,, 10	3				
,, 11	4				
,, 12	5				
,, 13	6	Starved			
,, 14	7	Fed on clean Dog 2054	+		Trypanosomes appeared in blood of Dog 2054 on the 31st day.
,, 15	8				
,, 16	9				
,, 17	10				
,, 18	11				
,, 19	12				
,, 20	13				
,, 21	14				
,, 22	15				
,, 23	16				
,, 24	17				
,, 25	18				
,, 26	19				
,, 27	20				
,, 28	21				
,, 29	22				
,, 30	23				

Table II—*continued*

Date	Day of expt	Procedure	Result: Positive	Result: Negative	Remarks
		Experiment 2067—*continued*			
May 1	24				
" 2	25				
" 3	26				
" 4	27	Fed on clean Dog 2054	+		Trypanosomes appeared in blood of Dog 2054 on the 31st day
" 5	28				
" 6	29				
" 7	30				
" 8	31				
" 9	32	Starved			
" 10	33				
" 11	34	Fed on clean Monkey 2131	+		Trypanosomes appeared in blood of Monkey 2131 on the 42nd day
" 12	35				
" 13	36	Starved			
" 14	37				
" 15	38				
" 16	39	Fed on clean Guinea-pig 2145		–	
" 17	40				
" 18	41				
" 19	42				
" 20	43	Starved			
" 21	44				
" 22	45	Fed on clean Duiker 2059	+		Trypanosomes appeared in blood of Duiker 2059 on the 60th day
" 23	46				
" 24	47				
" 25	48	Starved			
" 26	49				
" 27	50				
" 28	51	Fed on clean Monkey 2184	+		Trypanosomes appeared in blood of Monkey 2184 on the 63rd day
" 29	52				
" 30	53				
" 31	54				All flies dissected and three found infected
		Experiment 2394			
Aug 20 to Aug 26	1–6	40 flies fed on infected Rat 2389			
Aug 21	7	Starved			
Aug 22 to Oct 24	8–65	Fed on clean Dog 2404	+		Trypanosomes appeared in blood of Dog 2404 on the 65th day No flies dissected

Experiment 2067 was a successful experiment, as all the animals the flies fed on became infected with the exception of the guinea-pig, and it will be remembered that the guinea-pig was found to be refractory to this strain.

Experiment 2394 also infected a dog, but as none of the flies were dissected none were found infected

From these two experiments it would appear that a period of from 24 to

58 days may elapse before the cycle of development of the Naturally Infected Dog strain is complete in *G morsitans* and the fly becomes infective

Details of the Nine Negative Experiments

The following Table shows the method of procedure in carrying out the nine negative experiments In each of them laboratory-bred flies were used

Table III

Expt	Day of expt	Procedure	Remarks
1257	1-5 6 7-44	25 flies fed on infected Dog 690 Starved Fed on clean Dog 1318	All flies dissected, all negative
1499	1-3 4 5-48	26 flies fed on infected Rat 1218 Starved Fed on clean Dog 1500	All flies dissected, one found infected
1668	1-3 4-5 6-23	10 flies fed on infected Monkey 1630 Starved Fed on clean Monkey 1670	All flies dissected, all negative
1753	1-3 4 5-40	50 flies fed on infected Monkey 1534 Starved Fed on clean Monkey 1778	18 flies dissected, all negative
2018	1-8 9 10-30 31 32-45	55 flies fed on infected Rats 1985 and 2023 Starved Fed on clean Monkey 2056 Starved Fed on clean Dog 2112	18 flies dissected, 2 infected
2018A	1-8 9 10-35	40 flies fed on infected Dog 2054 Starved Fed on clean Dog 2172	36 flies dissected, 7 found infected.
2226	1-6 7 8-37	40 flies fed on infected Rat 2214 Starved Fed on clean Dog 2233.	8 flies dissected; 1 infected
2303	1-2 3-13 14 15-44	40 flies fed on infected Duiker 2059 Fed on infected Rat 2280 Starved Fed on clean Dog 2319	29 flies dissected; all negative
2433	1-3 4 5-38	20 flies fed on infected Rat 2425 Starved Fed on clean Dog 2435	15 flies dissected; all negative

RESULT OF THE DISSECTION OF THE INFECTED FLIES

Table IV—Laboratory-bred Flies Positive Experiments Time, in days, means the number of days which elapsed between the first infective feed and the death and dissection of the fly

Expt	Time, days	Proboscis	Proventriculus	Crop	Fore-gut.	Mid-gut	Hind-gut	Salivary glands
2067	42	−			+	+	+	−
2067	50	−	+ +		+	+	−	+ +
2067	51				+	+		−

In Experiment 2067, 30 flies were used, all were dissected and three found infected—10 per cent This experiment was carefully carried out from beginning to end, and yet only one fly was found with invasion of the salivary glands This one fly seems to have done all the mischief, infecting one after the other a dog, monkey, antelope, and finally another monkey There is some doubt about this last monkey, as the fly died 50 days after its first infective feed, and the monkey had only come into use the day before This particular fly, however, is reported not to have fed on that day, no fresh blood having been found in its intestine It may be that it attempted to feed and so infected the monkey, but was unable to draw blood If this fly did not infect the last monkey, it is difficult to explain its infection, as all the remaining flies were dissected on the 54th day and all found to be negative

In Experiment 2394 none of the flies were dissected

Table V—Laboratory-bred Flies Negative Experiments

Expt	Time, days	Proboscis	Proventriculus	Crop	Fore-gut	Mid gut	Hind-gut	Salivary glands
1499	11	−	+ +	−	+ +	+		−
2018	24	−	+ +		+ +	+ +	+ +	−
2018	30	−			+	+		−
2018A	13	−	−		+ +	+ +	+	−
2018A	14	−	−		+ +	+ +	+	−
2018A	15	−	−		+	+		−
2018A	17				+ +	+ +	+	−
2018A	18	−			+ +	+ +	+ +	−
2018A	35	−			+	+		
2018A	35	−			+	+		
2226	32				+ +	+ +	+	−

One hundred and eighty-five flies were dissected and 11 found infected—5·9 per cent In none was there found any development in the proboscis nor invasion of the salivary glands.

From an examination of these Tables it will be admitted, in spite of the paucity of the material, that the Naturally Infected Dog strain belongs to the same group as *T gambiense* and *T brucei vel rhodesiense* in regard to its mode of development in *G morsitans.*

The Type of Trypanosome found in the Infected Flies

A number of drawings of the developmental forms of the Naturally Infected Dog strain of trypanosome was made from the alimentary tract and salivary glands of the infected tsetse flies In the intestine the same type of trypanosome was found which has already been described and figured in previous papers * In the only infected fly which showed a development in the salivary glands, the trypanosomes were described in the living unstained preparations as being exceedingly numerous, small and active In the stained preparations the trypanosomes were seen to be typical "blood forms" and absolutely identical to those figured in the development of the trypanosome causing disease in man in Nyasaland† and also in that of *T brucei*, Zululand, 1913‡ It is therefore unnecessary to figure them again

Conclusion

The trypanosome of the Naturally Infected Dog strain belongs to the same group as *T gambiense* and *T brucei vel rhodesiense*, the trypanosome causing disease in man in Nyasaland, and is probably merely a weak strain of the latter species

* 'Roy. Soc. Proc,' B, vol 83 (1911)

† *Ibid.*, B, vol 87, p. 516

‡ *Ibid*, B, vol. 87, p 493

The Trypanosome causing Disease in Man in Nyasaland.—The Naturally Infected Dog Strain. Part IV.—*Experiments on Immunity.*

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INTRODUCTION.

The following experiments were undertaken to find out whether the Naturally Infected Dog strain of the trypanosome causing disease in man in Nyasaland would protect against the other strains. These different strains have been described in previous papers as the "Human," the "Wild Game," the "Wild *Glossina morsitans*," "Zululand, 1913," etc., and here they will be known by the same names. "Human" will therefore mean a strain of this species of trypanosome coming from man, "Wild *G. morsitans*" from a tsetse fly, and so on.

These immunity experiments were necessarily one-sided, as it was, with three exceptions, only animals which had recovered from the weaker Naturally Infected Dog strain which were available.

There are practically no recoveries from the Human and other strains. One goat apparently recovered from the Mzimba strain, and a goat, monkey and dog from the Wild *G. morsitans* strains, and are included.

There are, therefore, no completed cross-inoculation experiments—or at least only one unsatisfactory one, Experiment 17—as would have been carried out if material had been forthcoming.

It will be seen from the following experiments that the Naturally Infected Dog strain failed to immunise animals against the Human, Wild *G. morsitans*, and Zululand, 1913, strains, but it is not known whether these strains, on the other hand, would have immunised animals against the Naturally Infected Dog strain or not:—

Experiment 1, Dog 459, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 April 20	459	From Dog 317	Naturally infected Dog 48	19	R
Nov 22	459	From Guinea-pig 1333	Human	4	59

Remarks.—Dog 459, which had recovered from Naturally Infected Dog Strain 48, when inoculated with a Human strain died in 59 days.

Conclusion.—The Naturally Infected Dog strain does not protect against the Human Strain IV, Chipochola.

Experiment 2, Monkey 1792, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1913 Jan 22	1792	From Rat 1741	Naturally infected Dog 48	5	R
June 24	1792	From Rat 2167	Naturally infected Dog 2033	—	—
July 12	1792	From Rat 2235	Human	9	61

Remarks.—Monkey 1792, which had recovered from Naturally Infected Dog Strain 48, and proved immune to Naturally Infected Dog Strain 2033, succumbs to the Human strain.

Conclusion.—The Naturally Infected Dog strain does not protect against the Human strain, Yoramu.

Experiment 3, Monkey 1793, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1913 Jan 22	1793	From Rat 1741	Naturally infected Dog 48	5	R
June 24	1793	From Rat 2167	Naturally infected Dog 2033	—	—
July 12	1793	From Rat 2235	Human	5	9

Remarks.—Monkey 1793, which had recovered from Naturally Infected Dog Strain 48, and proved immune to Naturally Infected Dog Strain 2033, succumbs in nine days to the Human strain.

Conclusion.—The Naturally Infected Dog strain does not protect against the Human strain, Yoramu.

Experiment 4, Monkey 2164, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1913 May 14	2164	From Rat 2091	Naturally infected Dog 2033	—	—
June 14	2164	From Dog 2157	Naturally infected Dog 2033	—	—
July 31	2164	From Rat 2285	Naturally infected Dog 48	—	—
Dec 17	2164	From Rat 2437	Human	7	Alive 1 3 14

Remarks—Monkey 2164, which was proved to be immune against the Naturally Infected Dog Strains 2033 and 48, reacts readily to the Human strain

Conclusion—Immunity to the Naturally Infected Dog strain does not imply immunity to the Human strain, Dongolosi

Experiment 5, Dog 690, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 July 17	690	Naturally infected	Naturally infected Dog 690	?	R
Nov 22	690	From Rat 1492	Naturally infected Dog 48	—	—
Dec 20	690	From Dog 1675	Wild *G morsitans*	10	43

Remarks—Dog 690, which had recovered from Naturally Infected Dog Strain 690, and proved immune to Naturally Infected Dog Strain 48, succumbs in 43 days to the Wild *G morsitans* strain

Conclusion—The Naturally Infected Dog strain does not protect against the Wild *G morsitans* strain

Experiment 6, Dog 1530, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 Oct 29	1530	From Rat 1491	Naturally infected Dog 48	16	R
1913 March 21	1530	From Rat 1991	Naturally infected Dog 48	—	—
April 11	1530	From Guinea-pig 2034	Wild *G morsitans*	10	23

Remarks.—Dog 1530, after having recovered from Naturally Infected Dog Strain 48, and shown to be immune on reinjection, readily succumbs to one injection of the Wild *G morsitans* strain

Conclusion—The Naturally Infected Dog strain does not protect against the Wild *G. morsitans* strain

Experiment 7, Monkey 1534, Naturally Infected Dog Strain.

Date	Expt.	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 Oct 29	1534	From Rat 1491	Naturally infected Dog 48	6	R
1913 March 21	1534	From Rat 1991	Naturally infected Dog 48	—	—
April 11	1534	From Rat 2020	Wild *G morsitans*	6	17

Remarks —Monkey 1534, having recovered from Naturally Infected Dog Strain 48, and proved to be immune to the same strain, succumbs in 17 days to the Wild *G morsitans* strain

Conclusion —The Naturally Infected Dog strain does not protect against the Wild *G morsitans* strain

Experiment 8, Monkey 1630, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 Nov. 22	1630	From Monkey 1534	Naturally infected Dog 48	10	R.
1913 March 21	1630	From Rat 1991	Naturally infected Dog 48	—	—
April 11	1630	From Guinea pig 2034	Wild *G morsitans*	13	85

Remarks —Monkey 1630, having recovered from Naturally Infected Dog Strain 48, and shown to be immune on re-injection, succumbs to the Wild *G morsitans* strain

Conclusion —The Naturally Infected Dog strain does not protect against the Wild *G morsitans* strain

Experiment 9, Goat 427, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 April 20	427	From Rat 392	Naturally infected Dog 48	10	R.
1913 Jan 22	427	From Rat 1741	Naturally infected Dog 48	—	—
Feb 11	427	From Rat 1875	Naturally infected Dog 48	—	—
Feb 28	427	From Dog 1906 and Rat 1882	Zululand, 1913	38	54

Remarks —Goat 427 has recovered from Naturally Infected Dog Strain 48, and has shown no reaction to two re-injections, but when inoculated with *T brucei*, Zululand, 1913, takes the disease and dies in 54 days

Conclusion —The Naturally Infected Dog strain does not protect against *T brucei*, Zululand, 1913

Experiment 10, Goat 432, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 April 20	432	From Rat 892	Naturally infected Dog 48	26	R
1913 Jan 22	432	From Rat 1741	Naturally infected Dog 48	—	—
Feb 11	432	From Rat 1735	Naturally infected Dog 48	—	—
Feb 28	432	From Dog 1906 and Rat 1832	Zululand, 1913	31	143

Remarks.—Goat 432 has recovered from Naturally Infected Dog Strain 48, but succumbs to *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally Infected Dog strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 11, Sheep 456, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 April 20	456	From Rat 892	Naturally infected Dog 48	5	R
1913 March 21	456	From Rat 1991	Naturally infected Dog 48	—	—
1914 Jan 7	456	From Rat 2470	Zululand, 1913	10	Alive 1.3.14

Remarks.—Sheep 456 has recovered from Naturally Infected Dog Strain 48, but reacts when exposed to the virus of *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally Infected Dog strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 12, Dog 1253, Naturally Infected Dog Strain.

Date.	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 Sept 6	1253	From Rat 1218	Naturally infected Dog 48	6	R
1913 Jan. 4	1253	From Rat 1570	Naturally infected Dog 48	5	R
Jan 17	1253	From Rat 1734	Naturally infected Dog 48	—	—
Feb. 28	1253	From Dog 1906 and Rat 1832	Zululand, 1913	6	48

Remarks.—Dog 1253 has recovered from Naturally Infected Dog Strain 48, but succumbs to *T. brucei*, Zululand, 1913.

Conclusion.—The Naturally Infected Dog strain does not protect against *T. brucei*, Zululand, 1913.

Experiment 13, Monkey 1794, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1913 Jan 22	1794	From Rat 1741	Naturally infected Dog 48	--	—
Feb 28	1794	From Rat 1945	Naturally infected Dog 48	—	—
May 23	1794	From Monkey 2131	Naturally infected Dog 48	—	—
June 11	1794	From Monkey 2184	Naturally infected Dog 48	—	—
„ 24	1794	From Guinea pig	Zululand, 1913	9	42

Remarks—Monkey 1794 has been proved to be immune to Naturally Infected Dog Strain 48, but when exposed to the Zululand strain succumbs

Conclusion—Immunity to the Naturally Infected dog strain does not imply immunity to *T brucei*, Zululand, 1913

Experiment 14, Monkey 1798, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1913 Jan 22	1798	From Monkey 1680	Naturally infected Dog 48	—	—
Feb 28	1798	From Rat 1945	Naturally infected Dog 48	—	—
May 22	1798	From Monkey 2131	Naturally infected Dog 48	—	—
June 11	1798	From Monkey 2184	Naturally infected Dog 48	—	—
June 24	1798	From Guinea-pig 2225	Zululand, 1913	9	15

Remarks—Monkey 1798 has been proved to be immune to Naturally Infected Dog Strain 48, but succumbs to *T brucei*, Zululand, 1913

Conclusions—The Naturally Infected Dog strain does not protect against *T brucei*, Zululand, 1913

Experiment 15, Monkey 2161, Naturally Infected Dog Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days or recovery
1913 May 14	2161	From Rat 2091	Naturally infected Dog 2033	—	—
June 14	2161	From Dog 2157	Naturally infected Dog 2033	—	—
July 31	2161	From Rat 2285	Naturally infected Dog 48	—	—
Dec 17	2161	From Rat 2451	Zululand, 1913	7	Alive 1 3 14

Remarks —Monkey 2161, proved to be immune to Naturally Infected Dog Strains 2033 and 48, shows no immunity to *T brucei*, Zululand, 1913.

Experiment 16, Goat 639, Wild *G morsitans* Strain.

Date	Expt.	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 June 12	639	From Rat 543	Wild *G morsitans*	12	R
1913 Jan 22	639	From Rat 1741	Naturally infected Dog 48	—	—
Feb 11	639	From Rat 1735	Naturally infected Dog 48	—	—
Feb 28	639	From Dog 1906 and Rat 1832	Zululand, 1913	10	48

Remarks —Goat 639 has recovered from the Wild *G morsitans* strain, has shown no reaction when inoculated with Naturally Infected Dog Strain 48, but is killed in 48 days by *T brucei*, Zululand, 1913

Conclusion —The Wild *G morsitans* strain, combined with the Naturally Infected Dog strain, has no protective power against *T brucei*, Zululand, 1913

Experiment 17, Monkey 970, Wild *G morsitans* Strain.

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 July 24	970	From Rat 658	Wild *G morsitans*	8	R
1913 Jan 2	970	From Rat 1664	Wild *G morsitans*	—	—
„ 17	970	From Rat 1740	Wild *G morsitans*	—	—
Feb 4	970	From Rat 1814	Naturally infected Dog 48	9	R
„ 28	970	From Rat 1945	Naturally infected Dog 48	—	—
March 15	970	From Guinea-pig 1657	Human	9	71

Remarks—Monkey 970, after recovering from the Wild *G morsitans* strain, shows a reaction to Naturally Infected Dog Strain 48, and finally succumbs to a Human strain

Conclusion—The Wild *G morsitans* strain does not protect against the Naturally Infected Dog strain, nor does the combination of the two against the Human Strain V, Chibibi

Experiment 18, Dog 602, Wild *G morsitans* Strain

Date	Expt	Source of virus	Strain of trypanosome	Period of incubation, in days	Duration of disease, in days, or recovery
1912 May 31	602	Wild flies	Wild *G morsitans*	5	R
Nov 22	602	From Guinea pig 1838	Human	6	28

Remarks—Dog 602, which had recovered from the Wild *G morsitans* strain, when inoculated with a Human strain died in 28 days

Conclusion—The Wild *G morsitans* strain does not protect against the Human Strain IV, Chipochola.

Conclusions

1 The Naturally Infected Dog strain does not protect animals from the Human, Wild *G morsitans*, and Zululand, 1913, strains.

2 The Wild *G morsitans* strain and the Naturally Infected Dog strain do not protect animals from the Human or the Zululand, 1913, strain

3 The Wild *G morsitans* strain does not protect against the Human strain.

4 In spite of the damaging evidence of these experiments, the Commission still holds the opinion that the Naturally Infected Dog strain is a weak strain of the trypanosome causing disease in man in Nyasaland, *T brucei vel rhodesiense*

The Colouring Matters in the Compound Ascidian Diazona violacea, *Savigny.*

By Alfred Holt, M.A., D.Sc.

(Communicated by Prof. W. A. Herdman, F.R.S. Received May 12,—Read June 18, 1914.)

1. *Experimental Observations.*

The present investigation on the colouring matters of the compound Ascidian *Diazona violacea*, Sav. (= *Syntethys hebridicus*, Forbes and Goodsir), had its origin in an observation of Prof. W. A. Herdman while dredging in the neighbourhood of the Outer Hebrides in 1912.

Some specimens of this rare Ascidian were collected by Prof. Herdman, which showed whilst alive the green tint described by Forbes and Goodsir,* but on placing them in alcohol for purposes of preservation it was found that after a few hours the alcohol had acquired the original green colour of the Ascidian, while the organism itself had changed to violet, a shade nearly complementary to that of the living animal. A description of these specimens has already been published.†

During another expedition to the Hebrides in the summer of 1913, Prof. Herdman obtained so many specimens of this organism that it was possible to use some of the material for an examination into the nature of the green and violet pigments, but a complete study has been impossible owing to the minute quantity of pigment found in any one Ascidian colony, and to the fact that no fresh and living material was at my disposal. The green alcoholic solution obtained from the specimens collected in 1912 had been examined, and a brief account of the results was given in the above-mentioned paper in the Journal of the Linnean Society, but it will be useful to begin by recapitulating them here, and also to add some further information.

The green colour of the solution was not unlike that due to chlorophyll, and it exhibited well marked red dichroism. The absorption spectrum consisted of a broad band in the orange red, which was characterised by a more distinct edge towards the red than towards the yellow, and there was also practically complete absorption at the blue end of the spectrum. The band had the greatest intensity about $\lambda = 620\ \mu\mu$, and the absorption in the blue and violet began at $\lambda = 470\ \mu\mu$, and continued downwards.

Though not identical, this spectrum is not unlike that of true chlorophyll,

* 'Roy. Soc. Edin. Trans.,' vol. 20, p. 307.

† 'Linn. Soc. Zool. Journ.,' vol. 32, May, 1913.

in so far that the general absorption towards the violet begins at almost the same point, and that there is a very definite band in the orange red The two spectra are shown in the accompanying figure, where their points of agreement and disagreement are more immediately visible

In 1875, Sorby* obtained a green alcoholic solution from the Gephyrean worm *Bonellia viridis*, which, when examined spectroscopically, gave an absorption spectrum which also resembled chlorophyll in some respects In neutral solution there was a very pronounced band at $\lambda = 636\ \mu\mu$ and distinct bands at $\lambda = 587$, 520, and $490\ \mu\mu$, but he does not record any general absorption for $\lambda < 470\ \mu\mu$ His spectrum ("bonelleine") is also reproduced in the figure for the sake of comparison

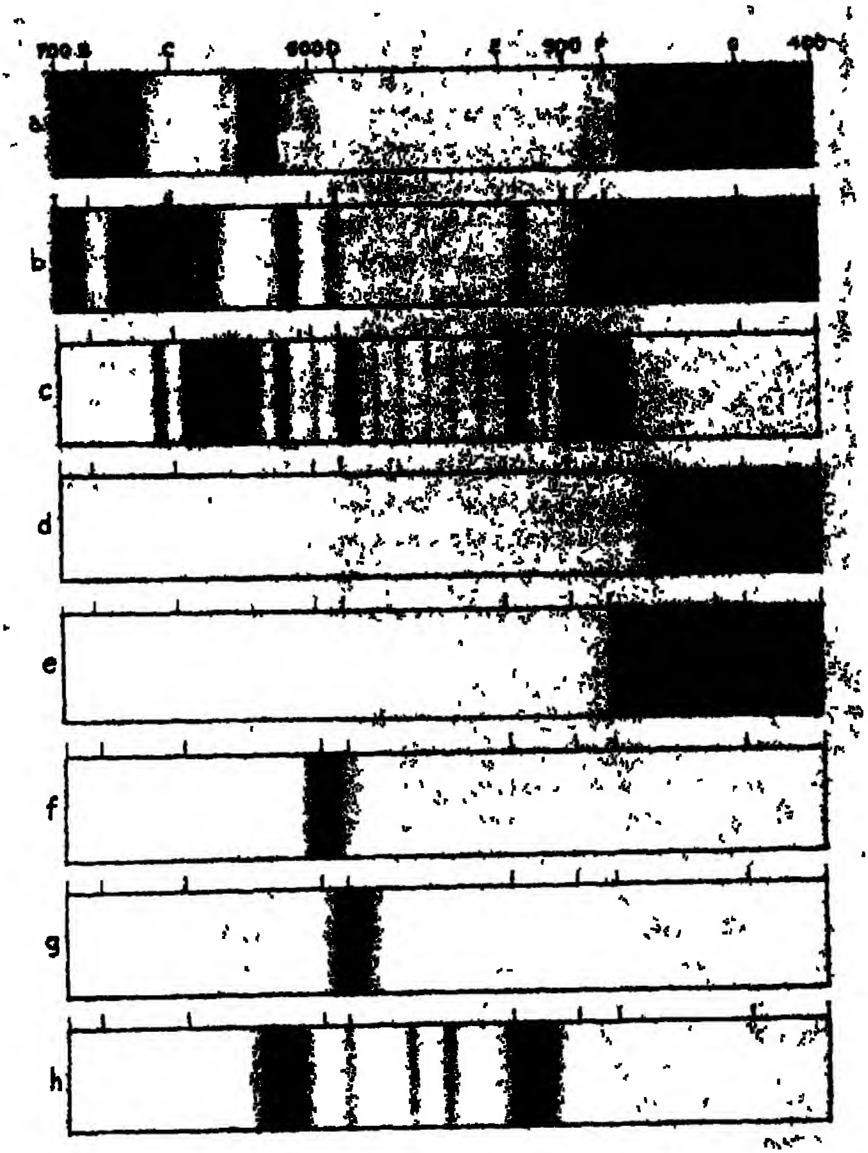
Judging solely from these observations, it appears very probable that the green solutions from *Diazona* and *Bonellia* contain either chlorophyll (for chlorophylls from different sources have not identical spectra), or some closely related chlorophyll body

On cutting a violet, alcohol-preserved specimen of *Diazona* in two, it was found that the tint only extended a short way (about 1 cm) beneath the surface, by far the larger mass of the colony (perhaps 20 cm across) remaining a pale greenish yellow The coloured outer portion of a specimen was therefore removed, and extracted with absolute alcohol A blue-green solution was slowly obtained, but only a very minute portion of the violet pigment appeared to pass into solution On cooling the alcoholic extract the blue-green colour became both paler and more yellow, though the original tint was restored on reheating, while after standing in the cold for some days a very small quantity of a substance having a violet tint identical with that observed in the outer portions of the colony was precipitated, the solution from which it had separated being now yellow-green Extraction of the inner portions of a colony with absolute alcohol gave a solution of an almost pure yellow tint, scarcely any trace of green being detectable by the eye No change took place either on cooling or after standing for some time

The spectrum of the blue-green solution appeared to be similar to that already described, there being absorption in the red, and general absorption in the blue and violet, but the yellow solution from the interior of the colony gave no distinctive band, but only a general absorption of the more refracted rays

On standing for several days these solutions became somewhat paler in tint, but attempts to concentrate the colour by distilling off the alcohol resulted in such turbidity that spectroscopic observations were impossible The original green solution obtained from the 1912 specimens has scarcely altered in tint (April, 1914), but on concentration it also becomes turbid

* 'Quart. Journ Microsc. Sci,' vol. 15, p 167



a Diazona (green solution) in alcohol
b Chlorophyll in alcohol
c Bonelleine, green neutral solution in alcohol Sorby
d Diazona (yellow solution) in alcohol
e Chlorophyll (yellow pigment) in alcohol
f Diazona (purple pigment) in acetylene tetrachloride
g Purpura (purple pigment) in acetylene tetrachloride
h Bonelleine, purple acid solution in alcohol Sorby

A few of the 1913 specimens had been preserved in formaldehyde solution instead of in alcohol These had retained their natural green colour, and on treatment with alcohol gave a pale yellow-green solution, the organism itself becoming practically colourless, no trace of purple being observed

This pale yellow solution showed a faint absorption in the blue and violet, but no band in the orange-red All the above greenish or yellow solutions were shaken with carbon disulphide to see whether any separation of the colouring matter could be effected The green 1912 solution after repeated shaking changed to a yellow, or brownish yellow, both the alcohol and carbon disulphide layers being coloured to about the same tint

The green solution from the outer portion of a colony gave a green carbon disulphide extract, the alcoholic solution becoming yellow. From the strength of the colour it appeared that there was far more green than yellow pigment present The yellow solution from the inner portion of the colony gave an exactly similar separation, but there appeared to be but little green pigment, as the carbon disulphide became only slightly coloured

The greenish yellow solution from the formaldehyde-preserved specimens was quite unaltered by shaking with carbon disulphide, this solvent seeming to dissolve no colouring matter

The green carbon disulphide extracts all showed an absorption band $\lambda = 620\,\mu\mu$ and general absorption for $\lambda < 470\,\mu\mu$, the yellow alcoholic portion exhibiting only a faint general absorption in the blue and violet. No satisfactory chemical observations could be made with any of these solutions Acids and alkalies gave no very characteristic reactions, as the addition of either merely caused the solutions to become somewhat more yellow This was particularly the case with alkalies, acids often appearing to have no action. Neutralisation of the alkaline solution did not restore the original colour. Acid or alkaline hydrogen peroxide was also without visible action

Saturated barium hydroxide solution gave a greenish precipitate with the green solutions, the supernatant liquid being yellow, while with the yellow solution the precipitate had a yellow tint, though the liquid was not completely discolored by the barium salt There was too little precipitate to try the action of an alcohol-glycerine solution of boric acid

The unsatisfactory nature of these reactions is mainly due, no doubt, to the great dilution of the solutions employed, but the fact that neither the specimens of *Diazona* nor the alcoholic extracts were fresh may be a contributing factor, for chlorophyll bodies are not very stable

It may be mentioned that fresh chlorophyll is altered in a non-reversible direction by acids, whereas the pigment from *Bonellia*, as described by

Sorby, changed to purple when strongly acidified, but regained its original shade on neutralisation.

From all the above observations it must be concluded that the green colour of *Diazona* probably results from some chlorophyll-like body. Though the spectra are not exactly those of ordinary plant chlorophyll there is quite a resemblance, and the association of separable green and yellow pigments from the alcoholic solution is also very suggestive.

If it is a chlorophyll body one is driven to the view that the green colour arises from a symbiotic alga, as chlorophyll does not appear to be a likely pigment for a marine animal. In a monograph on the compound Ascidian *Fragaroides aurantiacum*, by Charles Maurice,* the pigmentation of the test is described, and the author concludes that there the yellow pigment cells are in reality algæ (a *Protococcus*), which contain chlorophyll. He comments on the fact that these algæ when free show colours ranging from green to yellow, and that their cells during the period of reproduction resemble most closely the colour and structure of the globules in the test of the Ascidian. There are, however, three possible objections to this view. Firstly, *Diazona* has been collected from a depth of 60 fathoms, and it would appear to be most improbable that sufficient actinic light would penetrate to that depth to cause the formation of chlorophyll. Secondly, there is the evidence of Pizon† that in certain Tunicata which show very similar pigment cells to those of *Diazona* the yellow or yellow-green pigments result from the waste products of the organism, and are gradually excreted from its surface. Chlorophyll would hardly be a waste product. Thirdly, the pigment cells in *Diazona* are far smaller than the algal cells in known cases of symbiosis. The cells appear as minute spheres filled with one or more drops of an oily substance (judging by their high refractive index), and do not appear to show the structure of an algal cell. Until it is possible to work with some fresh, living colonies of *Diazona*, nothing more definite concerning the green pigment can be said than that it resembles chlorophyll in many respects, but is not identical with that ordinarily obtained from plants. It is, however, more like chlorophyll than the green pigment obtained by Sorby from *Bonellia*, and possibly represents algal cells.

Extraction with alcohol having shown that the purple pigment was all but insoluble, some 400 grm. of the organism were worked up on the lines employed by Friedlander in the case of the Mollusc *Murex brandaris*.‡

The material was first ground with sand and then digested for several

* 'Arch. de Biol.,' Liége, 1888.

† 'Compt. Rend.,' 1899, p. 395, and 1901, p. 170.

‡ 'Ber.,' 1909, p. 765.

hours with hot dilute sulphuric acid, fresh quantities of acid being used till the yellow tint at first imparted to it was no longer visible The mixture of animal matter and sand was then boiled with several portions of water and filtered. It was next extracted with alcohol Finally, it was treated in a Soxhlet with ethyl benzoate or acetylene tetrachloride These solvents acquired a fine blue colour, and exhibited a strong purple-red dichroism On cooling, the solutions gradually deposited a fine purple-black powder, which after recrystallisation and washing with ether did not greatly differ in tint from the violet colour of the Ascidian colony when preserved in alcohol When quite dry this purple powder had a distinct coppery lustre It was insoluble in water, alcohol, and ether, but soluble in aniline, pyridine, quinoline, nitro-benzene, ethyl benzoate, and acetylene tetrachloride, though the solubility varied with the liquid Thus, though easily soluble in hot acetylene tetrachloride, it was almost entirely reprecipitated on allowing the cooled solution to stand for some hours

In every case the solution was blue with a greenish shade when very dilute, changing to pure blue, and subsequently violet blue, on concentration When hot both the violet colour and the dichroism were more pronounced

The absorption spectrum was determined in both hot and cold ethyl benzoate and acetylene tetrachloride

The ethyl benzoate solution in the cold showed an absorption band with a maximum about $\lambda = 611\ \mu\mu$, though absorption began at $\lambda = 617\ \mu\mu$ When heated, the maximum was shifted to $\lambda = 605\ \mu\mu$, the band being very indefinite towards the green, the total absorption ranging from $\lambda\ 617\ \mu\mu$ to $\lambda\ 598\ \mu\mu$ In acetylene tetrachloride the maximum absorption both when hot and cold was shifted towards the green, the maximum when cold being $\lambda = 606\ \mu\mu$, and when hot $\lambda = 598\ \mu\mu$

The pigment dissolved in concentrated sulphuric acid to form at first a pinkish solution, which rapidly changed to a dirty purple colour. On standing, or more rapidly on warming, this colour changed to a brown tint with a green shade in it, or if sufficiently strong to a dull green The pink colour appeared to be of a transient nature, depending for its stability on concentration and low temperature

Addition of water to the cold acid solution precipitated the pigment, so it must be concluded that it does not form a soluble sulpho-salt, as is the case with indigo, but after heating, the addition of water caused the separation of a dull green flocculent precipitate, not the original pigment.

The colouring matter was insoluble in alkali, but gave a colourless solution with an alkaline reducing agent. Owing to the small quantity available it was impossible to try its action on cotton satisfactorily, but a cotton cloth in

which an Ascidian colony had been wrapped during preservation was found after drying and exposure to air and light to be dyed with a pale pink, not very fast, colour Qualitative examination showed the presence of a halogen, apparently bromine, for after treating a few milligrammes of the dyestuff by the Carius method a pale yellow silver precipitate was obtained which did not appreciably darken in sunlight and which was slowly soluble in excess of ammonia

The general behaviour of the colouring matter was thus seen to resemble a dibromindigo, which has been shown by Friedlander to be the dye in the case of the Mediterranean *Murex brandaris* The chief point of difference appeared to be the bluer shade of tint in all the solvents employed, the greater solubility in ethyl benzoate or acetylene tetrachloride, and except in strong, hot solutions the displacement of the maximum of the absorption band somewhat towards the red

As living specimens of *Murex brandaris* in quantity were not available for the sake of comparison, the pigment from the closely related British Mollusc *Purpura lapillus* was therefore examined

The purple pigment of this mollusc has already been studied by many chemists *

In the present instance the colouring matter from material collected at Port Erin, Isle of Man, was extracted in exactly the same way as described by Friedlander for *Murex brandaris*, and was obtained in a pure crystalline condition from solution in ethyl benzoate or acetylene tetrachloride

It will suffice here to say that its appearance and reactions agreed in every particular with the dye from *Murex brandaris* —66′ dibromindigo Solutions in various solvents were more red purple than those from *Diazona*, and its absorption band in hot acetylene tetrachloride gave $\lambda = 584\ \mu\mu$ The other three isomeric symmetrical dibromindigos have recently been described by Friedlander,† and their absorption and behaviour in concentrated sulphuric acid are given for reference from the above mentioned paper

Compound.	λ	Colour in concentrated sulphuric acid
	$\mu\mu$	
44′ dibromindigo	613	Blue
55′ ,,	621	Blue
66′ ,,	585	Dull violet brown
77′ ,,	606	Greenish blue (peacock blue)

* Bancroft, 'Philosophy of Permanent Colours,' 1803, Negri, 'Gaz. Chem. Ital.,' 1875; Schunk, 'Chem. Soc. Trans.,' 1879 and 1880, Letellier, 'Compt Rend,' 1889

† 'Ann. Chem,' vol. 388, p 23 (1912)

It will be observed that while the pigment from *Diazona* in some respects agrees with the 66′ body, in other respects it more resembles the 77′ isomer, which gives blue solutions in solvents, the colour being not unlike that of indigo. Possibly the *Diazona* pigment is some other isomer, or an indigo with a different number of substituted hydrogen atoms, but it is impossible to decide this point without far larger supplies of material.

For the sake of comparison, in the figure (p 229), the positions of the absorption bands for the violet or blue solutions obtained from *Diazona*, *Bonellia*, and *Purpura* are shown

2 *Origin and Formation of the Violet Pigment*

The experimental evidence so far available does not enable one to ascribe any certain origin to the violet pigment nor to account for its development in such different organisms as Mollusca (*Murex* and *Purpura*), Vermes (*Bonellia*), and Tunicata (*Diazona*) Nevertheless it may be useful to collect such evidence as there is at present to hand

In the case of *Murex brandaris* it seems to be well established that the colour has a photogenetic origin, but in *Murex trunculus* this is not the case, according to Negri (*loc cit.*). In *Purpura lapillus* the pigment is produced both by the action of sunlight, and by hydrochloric acid in the dark, this latter observation agreeing with the behaviour of *Bonellia viridis* according to Sorby Further, the pigments produced photogenetically in these organisms are uniformly insoluble in alcohol, while those resulting from the action of acids are soluble In the case of *Diazona* it is by no means certain that the pigment has a photogenetic origin Prof. Herdman has recorded the gradual production of violet colour in the living organism under the influence of sunlight, the original yellow-green tint changing first to blue green, then indigo blue, and finally a dull or dirty violet, but he is of opinion that this colour-change attends a moribund condition There is, however, no evidence that this change would not have taken place in the dark Natural violet-coloured specimens of the Ascidian have been obtained alive in the Mediterranean, near Naples, and also grey-green specimens which have remained unaltered after preservation in alcohol* but these natural violet specimens do not appear to be healthy, and hence the formation of the dyestuff may accompany or result from a metabolic change. In alcohol the colour is produced in the dark, for the specimens were placed in a closed tank immediately after they were collected Microscopic examination of an alcohol-preserved specimen shows the purple colouring matter apparently precipitated in the spherical pigment cells of the test, these cells in the inner

* See Herdman, 'Linn Soc Journ.,' 1913.

portion of the animal being filled, as already mentioned, with a bright yellow-green oily-looking substance

It is possible that the action of the alcohol may be merely that of precipitant, for if the dyestuff (which gives a blue solution) were dissolved in this oil the resultant colour would be the green of the living organism

The chloroplasts, as mentioned above, appear to contain a substance the solution of which in alcohol has an absorption spectrum resembling a yellow chlorophyll body, but it does not follow that they only contain this compound Some solvent for the indigo derivative may quite possibly be present in them as well

Now if this solvent is miscible with alcohol its removal would precipitate the colour body in its solid, violet-tinted form The alcoholic solution, however, would still have a greenish tint, since some of the pigment would dissolve in the alcohol-solvent solution, exactly as in the case of other three-component systems, and so add its blue colour to the yellow of the chlorophyll-like substance

Hence the presence of pigment in the alcoholic solution need not necessarily imply the existence of a second colour body soluble in alcohol, and this indeed is believed to be the origin of the traces found in some of the alcoholic extracts examined during this investigation It was remarked that the traces of colouring matter thus obtained were insoluble in absolute alcohol, a fact quite in accordance with the above view, since none of the natural animal solvent would then be present

It must, however, be pointed out that if the green colour of these extracts was due to the presence of a minute quantity of the violet pigment one would expect the spectrum to exhibit an absorption band about $\lambda = 606$ and not at $\lambda = 620\ \mu\mu$ It is of course a possibility that the animal solvent may shift the absorption band this amount towards the red, though this seems somewhat improbable in dilute solution in alcohol It is far more likely that the traces of violet pigment found in the alcoholic extract had their origin in a disintegration of parts of the organism during extraction from purely mechanical causes. Though the production of the violet colour could thus be explained when specimens are preserved in alcohol, this precipitation theory seems scarcely sufficient to explain all the observed facts According to this view the gradual production of the colour as observed by Prof Herdman in living specimens must arise from its production in such quantity that it can no longer be kept in solution by the solvent in the pigment cells, yet there is no evidence that there is more colouring matter present under these circumstances than when an ordinary green healthy colony is placed directly in alcohol. Further, it affords no explanation why the pigment is produced only

on the exterior, and not throughout the mass of the colony. It may also be remarked that a minute quantity of the pigment causes an intense coloration of its solvents, so much so, that if all the violet colouring matter in a colony was in solution during life the colour of the organism would almost certainly be a bright blue, not yellow green, as it would entirely mask the yellow of the chlorophyll body

The non-production of violet colour in the formaldehyde-preserved specimens is what one would expect from an indigo derivative, for the reducing action of the aldehyde would certainly produce colourless indigo-white derivatives, if indeed the whole molecule was not split up

The recorded phenomena can, however, be explained if we suppose that in the healthy animal the pigment is present dissolved in the pigment cells in its reduced condition as a chromogen Owing to its natural tendency to oxidation the animal by maintaining it reduced could use it as an oxygen carrier, and, since the only available oxygen is in the surrounding water, its presence would only be expected on the exterior of the colony, though the green-yellow chlorophyll-like pigment is present throughout its mass As soon as the animal became moribund or unhealthy metabolic processes would change and oxidation would begin, with the consequent production of colour. In the dead animal oxidation would be complete, and the colourless body converted into the violet pigment The same change would occur in alcohol, which by killing the animal would allow oxidation to proceed rapidly

The colour results no doubt from the action of an oxydase, which in the formaldehyde-preserved specimens would be destroyed, and hence no colour would result Until it is possible to experiment with living colonies one cannot express a definite view, but it appears more probable that some such series of changes as is outlined above takes place, rather than that the body in its fully oxidised condition is present in solution in the pigment cells of the live animal.

With only preserved colonies available it is not possible to prosecute further this enquiry as to these green and violet pigments or to express any opinion as to their possible relationship, as regards function in the organism, to those found in *Bonellia* and various Mollusca.

In conclusion my thanks are due to Prof. Herdman for providing several complete colonies of *Diazona* and the green alcoholic solutions obtained directly from the living organisms, and for suggesting to me that a chemical investigation might throw further light on the colour relations of the violet *Diazona violacea* and the green condition known as *Syntethys hebridicus*.

Some Accessory Factors in Plant Growth and Nutrition

By W B Bottomley, M.A, Professor of Botany, University of London, King's College.

(Communicated by Prof F W Oliver, F.R S Received May 29,—Read June 18, 1914.)

Recent research has demonstrated the importance of the presence of minute amounts of certain substances as accessory factors in normal dietaries of man and animals The most striking examples of the influence of these substances are seen in their curative effect on the diseases of beri-beri and scurvy, and their stimulative effect on the growth of young animals

Beri-beri is caused by the deficiency in a diet of polished rice of a nitrogenous substance, small amounts of which are essential for the metabolism of the nervous system The curative substance is found in rice husks, barley, wheat, lentils, yeast, egg-yolk, milk, etc, and is precipitated from an aqueous solution of an alcoholic extract of these bodies by phosphotungstic acid It is effective in very minute amounts, an addition to the diet of 0 02 grm of the active fraction of the extract curing polyneuritis (beri-beri) in pigeons

Scurvy also is caused by a diet adequate as regards proteins, carbohydrates and fats, but deficient in some constituent, small amounts of which are essential. This anti-scorbutic substance is found in lime-juice, fresh vegetables and fruits, and, like the curative substance of beri-beri, is precipitated by phosphotungstic acid

The special importance of small amounts of substances of unknown composition in the metabolism of growing animals has been demonstrated by the recent researches of Osborne and Mendel* and Hopkins† These investigations have shown that young rats, fed on a diet consisting of a mixture of pure proteins, carbohydrates, fats, and inorganic salts, failed to grow, but on the addition of a very small amount of certain substances obtained from milk growth was normal. Hopkins found that the fraction obtained from a phosphotungstic acid precipitation of proteid-free milk contained the active substance and gave excellent growth results As a result of his experiments he states that "the presence of minute traces of certain

* Osborne and Mendel, Carnegie Institution Publication No 156, Parts I and II, 1911.

† F. G. Hopkins, 'Journ. Physiol.' vol. 44 (1912).

organic substances are, without doubt, essential for the proper nutrition of growing animals"

Very little is known as to the nature and composition of these substances. Unfortunately, the active substance appears to be largely destroyed by chemical manipulations, and it is difficult to obtain sufficient to study its chemical constitution and properties. Funk,* by a complex fractionation of the phosphotungstic precipitate of anti-beri-beri substance, succeeded in isolating a substance, melting at 233° C, which in amounts of 0·02 to 0·04 grm cured polyneuritis in pigeons This substance he considered to be of the nature of a pyrimidine base Hopkins, however, states that the additions in his growth experiments were free from amino-acids, purine and pyrimidine bases. It is possible that these substances belong to a new group of nitrogenous compounds, which exist only in small amounts in food materials, but are so extremely active that minute quantities are sufficient to supply the needs of the organism

Although these substances have been found to occur chiefly in plants, there is no record of any investigations concerning the part, if any, they play in the metabolism of the plant itself.

During the summer of last year (1913) a number of experiments were made at the Royal Gardens, Kew, on a series of plants, to test the manurial value of Sphagnum peat which had been incubated with a mixed culture of aerobic soil organisms for a fortnight at a temperature of 26° C It had been discovered that by this bacterial treatment the humic acid in the peat is converted into soluble humates, and this bacterised peat, after sterilisation, forms an excellent medium for the growth and distribution of nitrogen-fixing organisms. As the experiments progressed it was evident that, in addition to the ordinary plant-food constituents, there was present in the bacterised peat a substance which stimulated growth in a remarkable manner. Further experiments showed that this substance was soluble in water, and was effective in very small quantities. Dr. Rosenheim, of King's College, found that seedlings of *Primula malacoides* potted up in loam, leaf-mould and sand, and treated twice with a water extract of only 0·18 grm. of bacterised peat, were, after six weeks' growth, double the size of similar untreated plants, and it was noted that flower production and root development were promoted equally with increase of foliage.

These results suggested that the growth-stimulating action of the bacterised peat might be due to the presence of a substance or substances similar in nature to the accessory food bodies concerned in animal nutrition.

* C. Funk, 'Journ Physiol.,' vol. 45 (1912–1913).

These accessory substances essential to animal nutrition are known to be soluble in water and alcohol, and, in order to ascertain as rapidly as possible whether there are present in the bacterised peat such water- and alcohol-soluble substances which have a similar effect on plant growth, an experiment was made to test the effect of an aqueous extract of the alcohol-soluble material of the bacterised peat on the growth and fixation of nitrogen by *Azotobacter chroococcum*.

The bacterised peat was extracted with absolute alcohol in a shaking machine for three hours, and the extract evaporated to dryness *in vacuo* The residue was taken up in warm distilled water, the liquid filtered, and the clear filtrate diluted until it contained the extract of 10 grm of peat per litre Portions consisting of 100 c c of this liquid were then transferred to each of 12 conical flasks, and the contents of six of the flasks boiled briskly over a Bunsen burner for five minutes, 100 c c. portions of distilled water were placed in each of six similar flasks, to each of the 18 flasks of the series were added 1 grm mannite, 0 2 grm. K_2HPO_4, 0 02 grm $MgSO_4$, and 0·2 grm $CaCO_3$, and each was inoculated with 1 c c. of a uniform suspension of *Azotobacter chroococcum*. The contents of two flasks from each of the three series of six were analysed at once to serve as controls, while the remaining four of each series were incubated for eight days at 26° C., at the end of which period they were analysed by the Kjeldahl process for their nitrogen content. The results are given in the following Table.—

Table I.

Series	Nitrogen content.			Nitrogen fixation.	Mean nitrogen fixation
		mgrm		mgrm	mgrm
I. Complete food	1 Control	0 4	Mean, 0·4 mgrm		
	2	0·4			
	3 Culture	4 6		4·2	
	4	4 4		4·0	3 8
	5	3·6		3·2	
	6	4 4		4·0	
II Complete food + alcoholic extract of bacterised peat	1. Control	2 6	Mean, 2 5 mgrm.		
	2.	2 3			
	3 Culture	20·7		18·2	
	4	20 5		18·0	18·0
	5.	19·9		17·4	
	6.	20·9		18 4	
III. Complete food + boiled alcoholic extract of bacterised peat	1. Control	2 3	Mean, 2 4 mgrm.		
	2.	2·5			
	3. Culture	19 6		17·2	
	4.	19·0		16·6	17·2
	5.	20·6		18·2	
	6.	19 4		17·0	

The more rapid growth of the organism in Series II and III was rendered apparent by the fact that a scum was visible on the surface of the liquid in each flask of these series after 24 hours, while the pellicle formed in Series I only after the lapse of 72 hours.

The results obtained indicated clearly that there is present in the bacterised peat a substance which stimulates plant growth, and that this substance is of a fairly stable nature is shown by the fact that almost as good results were obtained with the extract which had been boiled for five minutes as with the unboiled extract.

In order to test whether the active substance is present as such in the original peat, or whether it is produced in the bacterised peat as a result of treatment, an extract of the raw peat was made in precisely the same manner and in the same concentration as described for the bacterised peat Two series of cultures were prepared, the one containing complete food substances in distilled water, the other complete food in alcoholic extract of raw peat. The controls were analysed at once, while the cultures were incubated for eight days at 26° C as before. No increased growth was apparent in the cultures containing alcoholic extract of raw peat, while the results of analysis, as given below, indicate the absence of any stimulating substance —

Table II.

Series	Nitrogen content	Nitrogen fixation	Mean nitrogen fixation
	mgrm	mgrm	mgrm
I Complete food	1 Control 0 3 } Mean, 0 4 mgrm.		
	2 0 4		
	3 Culture 3 8	3 4	
	4 3·9	3 5	3 5
	5 3 8	3 4	
	6 4·0	3·6	
II Complete food + alcoholic extract of raw peat	1 Control 2 4 } Mean, 2 6 mgrm.		
	2. 2 8		
	3 Culture 4 0	1 4	
	4 4 6	2·0	1 9
	5. 4 6	2 0	
	6. 4·8	2 2	

The active substance is evidently produced in the bacterised peat as a result of treatment, and since this treatment consists essentially in the production of soluble humates by bacterial action, a test was made to ascertain whether the chemical production of soluble humates would be equally effective. Two equal portions of raw peat were saturated with solutions containing 1 per cent. of their weight of sodium carbonate, and were stirred at frequent intervals for

several hours One portion was allowed to dry slowly at room temperature, an alcoholic extract taken and evaporated *in vacuo* as before, the residue being made up in aqueous solution to a concentration of 10 grm. of carbonated peat per litre The other portion was leached with water until the washings were colourless, the liquid filtered, and the aqueous extract thus obtained was evaporated *in vacuo* to dryness The residue was extracted with alcohol, and the alcoholic solution again evaporated to dryness *in vacuo*, the residue being taken up with water, filtered, and the clear filtrate diluted to the proportion of the extract of 10 grm. of the original peat per litre The effect of both these extracts was tested on Azotobacter, three series of cultures being incubated—one containing complete food in distilled water, the second complete food in alcoholic extract of carbonated peat, and the third complete food in alcoholic extract of water-soluble substances from carbonated peat. Again no appreciable effect was observed on the growth of the cultures, while the results of the analyses as given below failed to reveal any stimulation of the organism —

Table III.

Series	Nitrogen content			Nitrogen fixation	Mean nitrogen fixation
			mgrm	mgrm	mgrm
I. Complete food	1	Control	0 4		
	2		0 5 (Mean, 0 5 mgrm)		
	3	Culture	4 8	4 3	
	4.		4·0	3 5	4·0
	5		4 4	3·9	
	6		4 8	4 3	
II Complete food + alcoholic extract of carbonated peat	1	Control	2 2		
	2		2 2 (Mean, 2 2 mgrm)		
	3	Culture	4 6	2 4	
	4		5·0	2 8	2 4
	5		4 4	2 2	
	6		4 6	2 4	
III Complete food + alcoholic extract of water-soluble substances from carbonated peat	1	Control	1 9		
	2		1 7 (Mean, 1 8 mgrm)		
	3.	Culture	4 4	2 6	
	4.		4 2	2 4	2 7
	5		4 6	2 8	
	6		5·0	3 2	

The results thus far obtained tend to prove that the active stimulant of plant growth which is present in bacterised peat does not exist as such in the raw peat, nor can it be liberated by a chemical production of soluble humates. It has been obtained only as a result of bacterial action.

Cooper and Funk* in 1911 showed that their curative substance was

* Cooper and Funk, 'Lancet,' p. 1267 (1911)

entirely precipitated by phosphotungstic acid from an aqueous solution of the dry residue from the alcoholic extract of rice polishings, and Hopkins* also states that he obtained the best results upon growing rats with the fraction from the crude phosphotungstic acid precipitate of protein-free milk. Consequently an experiment was made to determine how far the phosphotungstic acid fraction of the bacterised peat extract was effective in stimulating plant growth The bacterised peat was extracted with absolute alcohol as described above, and the alcohol evaporated off *in vacuo*. The residue was taken up in water, filtered, and to the filtrate sulphuric acid was added, until the concentration of the latter reached 5 per cent A slight precipitate of humic acid was filtered off, and to the filtrate an excess of 30-per-cent. solution of phosphotungstic acid was added. The whole was then left to stand overnight, when the liquid was decanted off through a filter, the precipitate repeatedly washed with a 5-per-cent. solution of sulphuric acid, and finally decomposed with an excess of baryta. The liquid was filtered off from the precipitate of barium phosphotungstate, and the filtrate, freed from the last traces of baryta by means of a very dilute solution of sulphuric acid, was evaporated to dryness *in vacuo* From 7 kgrm. of bacterised peat the amount of dry substance obtained from the phosphotungstic acid fraction amounted to 12·0096 grm., and since this was made up for experimental purposes into a solution containing the fraction from 10 grm. of peat per litre, the proportion of the dry phosphotungstic acid fraction in the final solution employed consisted of 17 parts per million. This fraction was tested upon wheat-seedlings in conjunction with Detmer's complete food solution. Ten seeds were germinated in clean sand in each of nine pots, which were arranged in three series of three pots each. Series I was treated with a complete food solution, Series II with complete food plus alcoholic extract from 10 grm. of peat per litre of solution, and Series III with complete food plus phosphotungstic fraction from 10 grm. of peat per litre of solution. The food solution employed contained nitrogen, phosphorus and potash, estimated as NH_3, P_2O_5, and K_2O in the proportion of 400, 200, and 1220 parts per million respectively, so that in addition Series III had 17 parts per million of dry substance obtained from the phosphotungstic fraction. Each pot was treated with 100 c.c. of its solution one week after sowing the seed, and the treatment repeated once weekly for five weeks, at the end of which period the plants were uprooted, washed, dried, and weighed. The results were as follows:—

* Hopkins, 'Brit. Med. Journ.,' vol. 2, p. 463 (1913).

Table IV.

Series	Weight of 30 plants	Increase over Series I
	grm.	per cent.
I Complete food solution	11·94	—
II " " " + alcoholic extract	14·46	21 1
III. " " " + phosphotungstic fraction	15 45	29 4

The results thus obtained indicate that the stimulative substance in bacterised peat is precipitated by phosphotungstic acid, and that this phosphotungstic fraction is quite as effective as the original alcoholic extract of the peat. Funk* found that, upon further fractionation of his phosphotungstic acid precipitate with silver nitrate and baryta and elimination of the reagents, he obtained a relatively pure crystalline substance, to which he gave the name "vitamine," and this he considered to be the specific curative substance. In order to determine how far the growth stimulant in bacterised peat resembled these so-called "vitamines," a further fractionation was carried out along the lines described in his paper. The phosphotungstic acid precipitate was decomposed, as before described, with baryta, and the last traces of baryta eliminated by means of sulphuric acid To the filtrate from the barium salt silver nitrate was first added, and then baryta, until no further precipitate was produced. The brownish precipitate was filtered off, well washed, suspended in dilute sulphuric acid, and decomposed with sulphuretted hydrogen. The filtrate from the silver sulphide was then exactly neutralised with baryta, the clear liquid filtered off from the precipitate of barium sulphate, and evaporated to dryness *in vacuo* The weight of dry substance obtained from the silver fraction from 7 kgrm. of bacterised peat amounted to 0·2452 grm, and, since this also was made up for experiment into a solution containing the silver fraction from 10 grm of peat per litre, this solution contained the dry substance from the silver fraction in the proportion of 0 35 part per million. This fraction was also tested, concurrently with the phosphotungstic acid fraction, upon wheat seedlings; 15 seeds were germinated in clean sand in each of nine pots, which were arranged in three series of three each. Series I was treated with complete food solution, containing nitrogen, phosphorus, and potash, estimated as NH_3, P_2O_5, and K_2O, in the proportion of 400, 200, and 1220 parts per million respectively Series II was treated with a similar solution, containing in addition 17 parts per million of the phosphotungstic fraction,

* Funk, 'Journ. Physiol,' vol. 45 (1912–1913).

and Series III with the complete food solution +0·35 part per million of the silver fraction The pots were first treated one week after sowing the seed, and after that each pot received once weekly 100 c c. of its food solution for seven weeks At the end of that period the plants were washed, dried, and weighed, and, after the gross weight had been taken, the plants were all dried in the steam oven at 100° C until their weight was constant. The results are as follows —

Table V

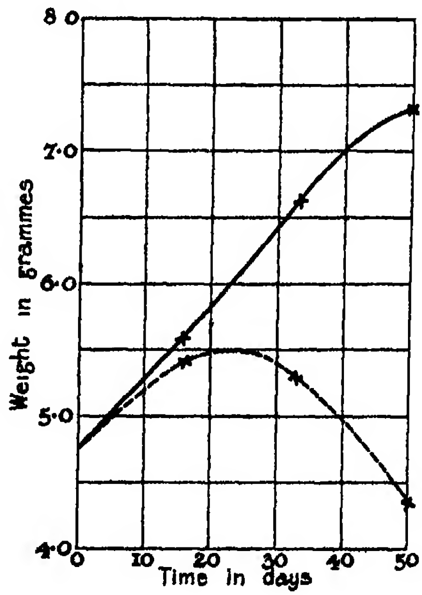
Series	Gross weight, 45 plants	Increase over Series I	Dry weight	Increase over I
	grm	per cent	grm	per cent
I Complete food	64 5	—	13 3480	—
II „ „ + phosphotungstic fraction	96 8	50 0	16 3818	22 7
III „ „ + silver fraction	96 5	49·6	15 7148	17 7

The silver fraction from the bacterised peat extract, corresponding with the "vitamine" fraction of Funk, having thus given results approaching those of the phosphotungstic fraction, a preliminary investigation was made to test its effect on the growth of wheat seedlings in water culture Two sets, each consisting of 18 similar seedlings, were carefully selected, each set being originally of equal weight, viz, 4 73 grm Each set was divided for purposes of water culture among three similar bottles of 200 c.c capacity, six plants being inserted through notches in the corks of each bottle, so that the roots dipped into the culture solution The three bottles of set I were filled with a nutrient solution of pure salts in physiologically pure distilled water, in which the proportions of NH_3, P_2O_5 and K_2O were 400, 200, and 1220 parts per million respectively; while those of set II contained a precisely similar solution which had received, in addition, 0·35 part per million of the silver fraction of bacterised peat extract. The bottles were aerated daily, and the solutions changed twice a week, while at the end of every 16 or 17 days the plants were taken from the jars, moisture removed from their roots by means of blotting paper, and weighed. The results obtained are shown in Table VI

The change brought about by the addition of the silver fraction is represented by the accompanying curves, in which the dotted line represents the change in weight of the series in pure food, while the unbroken line shows the progressive increase in weight obtained upon the addition of this substance.

Table VI.

Series	Weight of set of 18 plants		Percentage increase on original weight
		grm	per cent
I Pure food solution	Original weight	4 73	—
	After 16 days	5 42	14 7
	After further 17 days	5 29	11 8
	" " "	4 33	−8 4
II Pure food solution + silver fraction from bacterised peat	Original weight	4 73	—
	After 16 days	5 57	17 7
	After further 17 days	6 65	40·6
	" " "	7 33	54 9



Up to a certain point the two series of plants increased in weight to an almost equal extent, but beyond this point the seedlings growing in pure food solution appeared to be unable to utilise the food elements supplied to them; a condition which was apparently corrected by the addition of the silver fraction

Experimenting with guinea-pigs in 1909, Fürst* demonstrated that seeds of barley, oats, peas and flax contained no curative substances for scurvy, but that during the germination of these seeds anti-scorbutic substances developed, which were quite as effective as extracts from green vegetables.

* Fürst, 'Zeitschr. f. Hyg. u Infekt.,' vol. 72, p. 121

These facts indicate the possibility of the development, during germination, of special growth substances which enable the young embryo to utilise the food material present in the seed. If this is so, the removal of the source of these growth stimulants by the cutting off of the seed as soon as possible after germination should render the effect of an addition of such substances in the food solution all the more marked. In order to test this hypothesis, two series of wheat seedlings, similar to those used above, but in a rather younger stage, were taken, and before the removal of their seeds the two sets were of equal weight, viz 3 97 grm Their seeds were carefully removed, injury to the plants being avoided, and after this process the two sets weighed respectively 3·2 and 3 17 grm These were treated in precisely the same manner as before, the first set being given complete food salts, and the second food salts with the addition of the silver fraction from bacterised peat. The weights of the two sets at various dates are shown in the following Table —

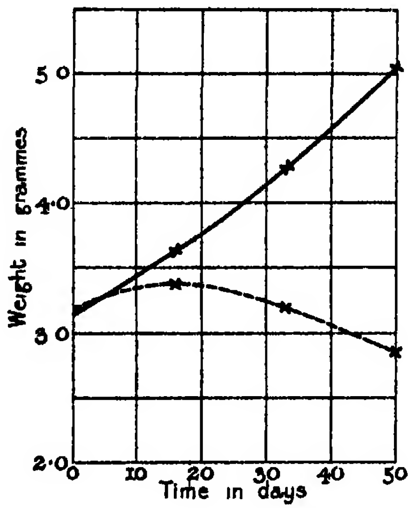
Table VII.

Series	Weight of set of 18 plants		Percentage of increase in weight
		grm.	per cent.
I Complete food solution	Original weight	3 2	—
	After 16 days	3 37	5 3
	After further 17 days	3 20	0·0
	„ „ „	2 85	−10 9
II Complete food solution + silver fraction from bacterised peat	Original weight	3 17	—
	After 16 days	3·63	14 5
	After further 17 days	4 29	35 3
	„ „ „	5·05	59·3

The following diagram shows the variation in weight of the seedlings throughout the experiment, the dotted curve representing the series in pure food, while the unbroken curve shows the effect of the addition of the silver fraction

These results indicate that during the germination of wheat seeds certain substances are formed which enable the young embryo to utilise the food materials present. The supply of these substances formed by the seed during germination is sufficient to establish the embryo as an independent seedling, then some other source is necessary. It has been shown that these accessory food substances are produced when peat—decayed vegetable matter—is acted upon by certain soil bacteria, and the natural inference is that during the bacterial decomposition of organic matter in the soil, that is, during humus formation, these substances are formed, hence the beneficial effect on crops of

farmyard and other organic manures The specific action of these accessory substances is not known They may be concerned in the metabolism of



phosphorus, they may act as catalytic agents, or may be a definite constituent of plant food—a "bau-stein"

Experiments to test these various hypotheses are in progress.

In conclusion I wish to acknowledge my indebtedness to Miss F. A. Mockeridge, B.Sc, for her valuable help in the chemical part of this investigation

Further Observations on the Changes in the Breathing and the Blood at Various High Altitudes.

By Mabel Purefoy FitzGerald

(Communicated by J S Haldane, F R S Received June 2,—Read June 25, 1914)

In a previous investigation carried out in connection with the Anglo-American Pike's Peak Expedition (1911), the changes in the breathing and the blood at high altitudes were recorded at atmospheric pressures ranging from 625 to 458 mm of mercury Lack of time prevented further observations being made, and in the graphic representation of the gases of the alveolar air and of the percentage of hæmoglobin in the blood subsequently published,* the supposed values for atmospheric pressures ranging from 625 to 760 mm of mercury were indicated by a broken line

To complete the records, experiments were made by me in North Carolina, U S A., during the months of July, August, and September of 1913. Three localities were chosen in the Southern Appalachian chain, approximately between 35° and 35° 6′ N latitude, and 82° 5′ and 83° 25′ W longitude Highlands (altitude 3850 feet), the highest village east of the Rocky Mountains, situated in the Blue Ridge Mountains, Waynesville (altitude 2645 feet) in the Balsam Mountains, and Asheville (altitude 2210 feet) situated in the valley of the French Broad River, with the Blue Ridge Mountains lying to the south and east, and the foot-hills of the Unaka Mountains to the west and north.

Experiments were made with 43 residents Care was again taken to exclude the unhealthy, and those who, on account of recent change of abode, might be unacclimatised Observations were also made on myself at each locality visited

The research is based upon 206 CO_2 determinations and 52 hæmoglobin percentage determinations. The subjects were adult men and women, of from 18 to 70 years of age The number of subjects corresponding to each decade, or part thereof, were as follows :—

Age.	Number
Between 15 and 19 years	8
„ 20 „ 29 „	15
„ 30 „ 39 „	14
„ 40 „ 49 „	1
„ 50 „ 59 „	2
„ 60 „ 69 „	4

* 'Phil. Trans.,' B, vol. 203, pp. 351–371.

In the majority of cases, the subjects were natives of the respective localities With the exception of two subjects, one of whom had been at sea-level three weeks prior to the experiment, and the other, one month before, at an altitude of about 1000 feet, no subject had left the place at which he or she was living for a considerable time

The methods of determining the alveolar CO_2 percentage, and of calculating the alveolar CO_2 and O_2 pressures, were the same as in the previous investigation

A carefully standardised Gowers-Haldane hæmoglobinometer was used for determining the hæmoglobin percentage, and pure CO was used for saturating the blood solution At Asheville and New York the readings for the barometric pressure were obtained from the local offices of the United States Weather Bureau, at Highlands and Waynesville readings were taken from an aneroid barometer compensated for temperature and checked by comparison with the readings of the Weather Bureau

The altitude records were taken from the bench marks of the United States Geological Survey, with the exception of that for Highlands, in which case the elevation recorded (3850 feet) is for the Highlands Camp Sanatorium, where the experiments were made, and not for the village proper

The mean values for the hæmoglobin percentage, the alveolar CO_2 percentage and pressure, and the calculated alveolar oxygen values for men, at altitudes varying from 658 to 711 mm of mercury are given in Table I, and similar values for women in Table II Normal mean values for near sea-level (Oxford) are included for comparison in each table

The results obtained in the present and the former investigation are indicated graphically in Charts I and II

It will be seen that taken in conjunction with the barometric pressure the values for the alveolar CO_2 and O_2 pressures decrease progressively with the increase of altitude Since the CO_2 values, with the exception of those for Waynesville, correspond closely with the supposed values indicated by the graph in the preceding paper* additional support is given to the statement then made, that the lowering of the CO_2 pressure is in direct proportion to the diminution of the barometric pressure and amounts to about 4 2 mm or 10 5 per cent. of the sea-level value for each 100 mm of diminution of barometric pressure. There is a corresponding progressive fall in the oxygen pressure of about 16 mm., or 16 per cent. of the sea-level value for each 100 mm. fall in the barometric pressure

In each of the two charts a curve is also plotted, as in the former paper,

* *Ibid.*, Chart 1

Table I.—Mean Results Obtained for Men

Locality	Altitude in feet.	Mean barometric pressure	CO_2							O_2		Hæmoglobin			
			Percentage in dry alveolar air	Pressure in alveolar air saturated at 37° C.			Number of subjects and of determinations.			Calculated mean percentage in dry alveolar air	Calculated mean pressure in alveolar air saturated at 37° C	Percentage.			Number of determinations
			Mean.	Mean	Max.	Min	Subjects.		Determinations			Mean	Max	Min.	
		mm. Hg													
Highlands (Camp Sanatorium)	3850	658	5 62	34 8	39·2	31·0	10		34	14·23	86·9	110 4	116	99	7
Waynesville ..	2645	691 (uncorrected)	5·24	33·8	37·0	31 3	13		40	14·67	94 5	108·6	117	97	9
		696 (corrected 0·5 cm.)	5·24	34·0	37 3	31·6	13		40	14·67	95 4	108·6	117	97	9
Asheville ..	2210	711	5·76	38·5	41·7	33 5	6		18	14·05	93 2	106·8	112	98	4
Oxford,* near sea-level	200	748	5·59	39·2	44·5	33 6	27		130	14 28	99·96	100·0†	113	87	26

* See FitzGerald and Haldane, 'Journ. Physiol.,' vol. 32, p 486 (1905)

† See Haldane, 'Journ Physiol.,' vol. 26, p 503 (1901)

Table II.—Mean Results Obtained for Women.

Locality	Altitude in feet	Mean barometric pressure.	CO_2						O_2		Hæmoglobin.			
			Percentage in dry alveolar air	Pressure in alveolar air saturated at 37° C			Number of subjects and of determinations		Calculated mean percentage in dry alveolar air	Calculated mean pressure in alveolar air saturated at 37° C	Percentage			Number of determinations
			Mean	Mean	Max.	Min	Subjects	Determinations			Mean	Max	Min.	
		mm. Hg												
Highlands (Camp Sanatorium)	3850	660	5·19	32·0	35·3	27·2	7	23	14·75	90·4	91·8	95	90·5	7
Waynesville	2645	688 (uncorrected)	4·81	30·8	31·7	29·8	2	6	15·20	97·4	97	99	95	2
		698 (corrected 0·5 cm.)	4·81	31·0	31·9	30·1	2	6	15·20	98·1	97	99	95	2
Asheville	2210	710	4·91	32·5	35·1	30·3	5	22	15·06	100·0	97·8	104	95	4
Oxford,* near sea-level	200	749	5·17	36·3	41·0	30·4	32	138	14·77	103·6	89·0†	96	81	12

* See FitzGerald and Haldane, 'Journ. Physiol.,' vol. 32, p. 486 (1905).
† See Haldane, 'Journ. Physiol.,' vol. 26, p. 486 (1901).

showing the values for the barometric pressure when the mean temperature of the air column between sea-level and the heights indicated is assumed to be 15° C

For the calculated values and graphic representation of the percentage

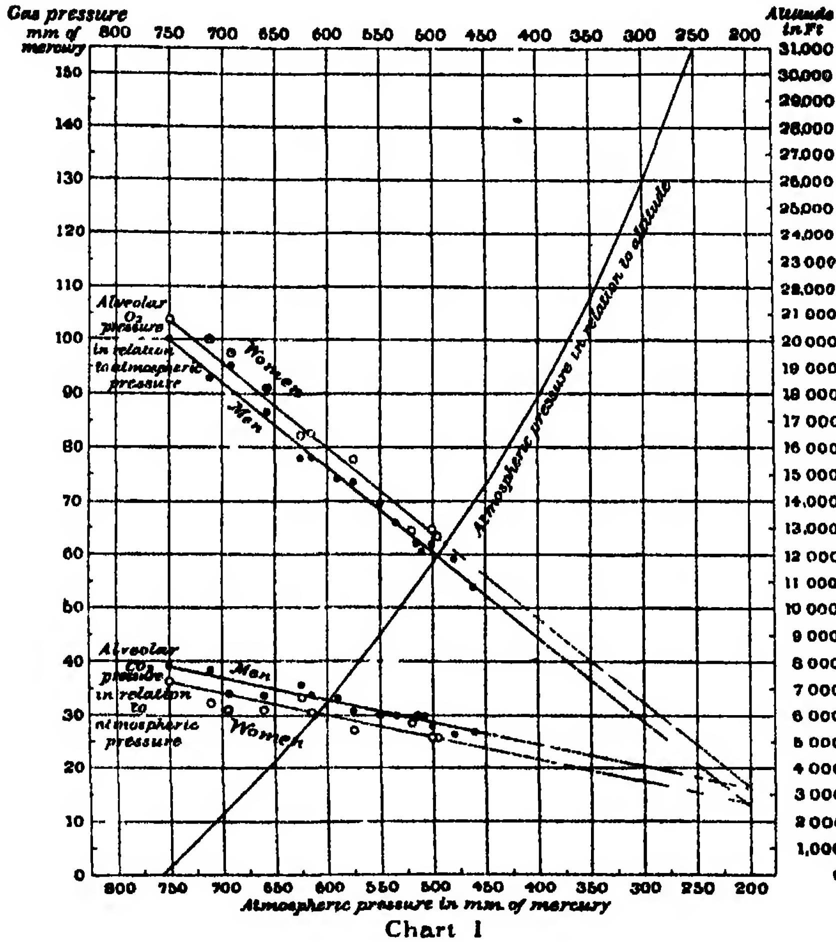


Chart I

composition of the alveolar gases at atmospheric pressures ranging from 760 to 250 mm. of mercury the reader is referred to the earlier paper *

The idea was put forward in that paper† that at atmospheric pressures greater than 625 mm of mercury the straight line representing the CO_2 tension might perhaps be replaced by a curve, and that this would flatten as

* *Ibid.*, pp. 359–360.
† *Ibid.*, p 360

a pressure of 760 mm. of mercury was approached, and continue as a straight line parallel to the abscissæ with increased pressure If such alteration occurs, it is evident that it can only be at pressures higher than 760 mm. of mercury. From the earlier experiments of Haldane and Priestley,* Hill and

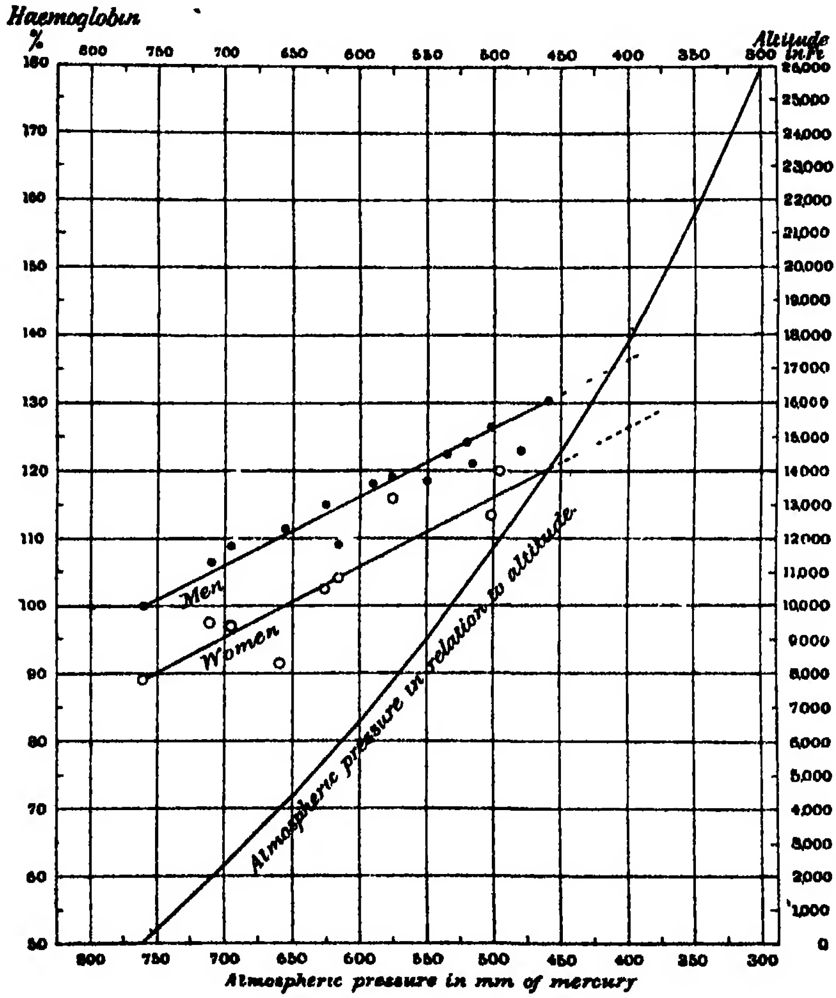


Chart II.

Greenwood,† and Haldane and Boycott,‡ we know that on short exposure to increased pressure the alveolar CO_2 remained at normal value, even up to

* 'Journ. Physiol.,' vol. 32, p. 225 (1905).
† 'Roy. Soc. Proc.,' vol. 70, p. 455 (1906).
‡ 'Journ. Physiol.,' vol. 37, Nos. 5–6, p 355 (1908).

seven atmospheres in the case of Greenwood. With long exposures, however, the result may well be different

The percentage of hæmoglobin in the blood in men increased progressively with the fall in barometric pressure. The mean percentage values correspond closely with the supposed values at similar pressures indicated in Chart III of the previous paper* and support the statement there made† that "for every 100 mm fall of atmospheric pressure, there is an average rise of about 10 per cent in the hæmoglobin." Similar, but less regular, increase occurred in the percentage of hæmoglobin in the blood of women An unusually low value (91·8 per cent) was recorded at Highlands Insufficient data together with varying physiological condition may account for the irregularity in the records for women

At each locality greater uniformity in the individual determinations was met with than in the previous investigation at the higher altitudes At Waynesville and Asheville, in four of the five men subjects aged between 52 and 64 years, the hæmoglobin percentage was less than 100, which possibly indicates that the compensatory increase of hæmoglobin in the blood wanes with age at the less high altitudes

With regard to the influence of age on the fall in the alveolar CO_2 pressure, the tendency previously noticed‡ for the fall to be less in those under 30 than at later age periods was again observed. Only one subject complained of the ill-effects of living at high altitudes (3850 feet) A marked difference was observed in the degree of "nervousness" in the subjects, this being much less than in residents at altitudes of 5000 feet and over.

Observations were made upon myself at each locality (see Table III), 24 hours were allowed to elapse before the alveolar CO_2 determinations were made My mean normal alveolar CO_2 pressure at Oxford and at New York is 34 mm of mercury (barometric pressures respectively 753 and 759 mm. of mercury) The journey of about 33 hours to Highlands (altitude 3850 feet) was made direct from New York, where the previous nine months had been spent At Highlands, a stay of over eight weeks was made. During the last two and a half weeks I slept at a point about 400 feet higher than the sanatorium, where work was conducted during the day, and through the eighth week was more or less stationary at the higher altitude (4250 feet). Contrary to the former experience at altitudes of 5000 feet and higher, the alveolar CO_2 pressure did not fall, but remained, with slight variations, at the normal value for sea-level (34 mm. of mercury). In consequence, the alveolar

* *Ibid*, p 362.
† *Ibid.*, p. 361.
‡ *Ibid*, p. 365.

Table III.—Mean Results obtained for M. P. F. G.

Locality	Altitude in feet	Mean barometric pressure	CO_2					O_2		Hæmoglobin			
			Percentage in dry alveolar air Mean	Pressure in alveolar air saturated at 37° C			Number of determinations	Calculated percentage in dry alveolar air Mean	Calculated pressure in alveolar air saturated at 37° C Mean.	Percentage			Number of determinations
				Mean	Max	Min				Mean	Max	Min.	
		mm Hg.											
Highlands (Camp Sanatorium)	3850	663	5 57	34 3	35·6	33 3	31	14 28	87·9	94	98	91	5
Waynesville	2645	689 (uncorrected)	5 39	34·6	34 8	34 4	4	14 50	93 1	93	—	—	1
		694 (corrected 0 5 cm)	5 39	34·9	35 1	34·7	4	14 50	93 8	93	—	—	1
Asheville	2210	708	5·20	34 4	34·8	33 7	8	14 73	97 3	96	—	—	1
Oxford*	200*	753	4·81	34·0	Records not		available	15 19	107 2	89	—	—	
Sea-level, New York	Sea-level	759	4 79	34 1	35 6	33 1	12	15 22	108 3	89	92	87	4

* See FitzGerald and Haldane, 'Journ Physiol,' vol 32, p 486 (1905)

oxygen pressure was lower than it otherwise would have been In general I was not conscious of being at a higher altitude than usual, but signs of being at a physiological disadvantage, due to the low alveolar oxygen pressure, were manifested by the constant feeling of great fatigue and, except during the stay at 4250 feet, when work was in part lessened, by poor and unrefreshing sleep *

From Highlands the journey was made via Lake Toxaway (seven hours' drive and thence by rail) to Waynesville (altitude 2645 feet), one night being spent on the way at Asheville (altitude 2210 feet). Four days were spent at Waynesville (2645 feet) Stormy weather prevailed The mean alveolar CO_2 pressure was found to be 34 6 mm of mercury with a barometric pressure of 689 mm of mercury (corrected barometric pressure 694 mm CO_2 pressure 34 9 mm. of mercury) At Asheville (altitude 2210 feet), where a week was spent after leaving Waynesville, the mean CO_2 pressure in the alveolar air was found also to be within the variation of normal sea-level values, *i.e* 34 4 mm. of mercury (barometric pressure 708 mm of mercury)

The return to sea-level (New York) was made direct from Asheville, a journey of 22 hours and involving a change of altitude of slightly over two thousand feet. Fourteen hours after arrival, the alveolar CO_2 pressure was found to be 33 1 (barometric pressure 766 mm of mercury), a slightly lower value than usual. On the third day at sea-level the CO_2 pressure was 34·0 mm of mercury. The values obtained during the subsequent three weeks varied from 33 2 mm. of mercury to 35 6 mm of mercury, the normal mean value obtained for New York being 34·1 mm (mean barometric pressure 759 mm. of mercury)

Thus at altitudes ranging from 4000 feet to 2000 feet, and at barometric pressures ranging from 663 to 708 mm. of mercury, there appears to be no respiratory reaction in M. P. F. G. Entirely different behaviour of the respiratory centre was therefore met with below 4000 and above 5000 feet, for in the latter experience (5000–14,000 feet),† although the response of the respiratory centre to want of oxygen was slow, it was nevertheless apparent. From the present series of experiments, and in spite of a stay of eight weeks at 3850 feet, the alveolar CO_2 pressure remained at sea-level value (34 mm. of mercury) at barometric pressures ranging from 759 to 663 mm. of mercury

The sea-level value of 34 mm. of mercury falls within the lower limits of

* In spite of this, however, there was a general improvement in condition as evidenced by increase in bodily weight. Excellent food was provided at the sanatorium, and the daily consumption of food was greater than usual. It must be borne in mind that fatigue was easily produced since I had been weakened during the previous winter by a chronic Staphylococcus infection.

† *Ibid.*, p 367.

alveolar CO_2 pressure observed for normal women. Whether the fact of my CO_2 pressure remaining at the normal value in spite of the barometric pressure varying from 759 to 663 mm of mercury is an idiosyncrasy, or an indication that with persons in whom the alveolar CO_2 pressure is naturally low a marked decrease of barometric pressure (*i e* more than 100 mm of mercury) is required to produce a lowering of the threshold value of the CO_2 pressure, can only be determined by further experiments.

In contrast to the absence of change in the alveolar CO_2, the hæmoglobin in M P F. G rose, as before,* with decreased barometric pressure and fell as sea-level pressure was approached From an initial value of 89 per cent at New York, the hæmoglobin had after three days at Highlands (altitude 3850 feet, barometric pressure 668 mm of mercury) risen to 96 per cent It oscillated during the following four weeks between 91 and 93 per cent and was 98 per cent during the eighth week, after a fortnight had been spent at an altitude of 4200 feet. At Waynesville (altitude 2645 feet) it fell to 93 per cent, and was again 96 per cent at Asheville (altitude 2210 feet and barometric pressure 708 mm. of mercury) Three days after the return to New York a lower value than usual was recorded, 87 per cent The hæmoglobin reached 92 per cent a few days later, and then fell to the normal value of 89 per cent

Conclusions.

1 In persons acclimatised at altitudes up to 3850 feet, the partial pressure of CO_2 in the air of the lung alveoli is invariably lower than at sea-level, so that the lung ventilation is correspondingly increased The results of the present investigation are in accord with those obtained with persons acclimatised at altitudes of 5000 to 14,000 feet, and support the conclusion previously published that "the lowering of the CO_2 pressure is in direct proportion to the diminution of the barometric pressure, and amounts to about 4 2 mm or 10 5 per cent of the sea-level value for each 100 mm of diminution of barometric pressure"

2 It is again found that in women, as at sea-level, the alveolar CO_2 pressure is about 3 mm lower than in men

3 As at higher altitudes, in persons acclimatised at altitudes up to 3850 feet, the percentage of hæmoglobin in the blood is increased. The present observations support the view previously published that "for every 100 mm fall of atmospheric pressure the percentage of hæmoglobin in the blood is increased by about 10 per cent of the normal value for men at sea-level"

* *Ibid.*, p 369

In women, as at sea-level, the values are about 11 per cent. lower than for men, but greater irregularity is observed.

Graphic representations and tables of the results are given To render possible a complete survey of the alveolar gas pressures and the hæmoglobin percentages recorded for acclimatised persons at varying atmospheric pressures and heights above sea-level, the values previously published* are included in the graphs

In conclusion, I wish to express my cordial appreciation of the kind help and hospitality received during the investigation. My thanks are specially due to Dr. Mary Lapham, of Highlands, Dr Stokes and Mr J Tull, of Waynesville, and to Dr George Purefoy and Messrs. Taylor and Johnstone (U S Weather Bureau), of Asheville

My sincere thanks are also due to Dr. J S Haldane for his advice and for the loan of standardised instruments, and to Prof Yandell Henderson, of Yale University, for the further loan of apparatus.

Constancy of the Optimum Temperature of an Enzyme under Varying Concentrations of Substrate and of Enzyme.

By Arthur Compton, Imperial Cancer Research Fund

(Communicated by Sir J R. Bradford, K.C.M G, Sec. R.S. Received June 10,—Read June 25, 1914)

In a recent paper† a new enzymic relation is recorded. For the enzymic hydrolysis of salicin—by the enzyme which Gabriel Bertrand and the author‡ have named *salicinase*—it is found that, in an action of fixed duration,§ the temperature of greatest activity of the ferment is always the same, whatever the dilutions of substrate and of enzyme adopted for the determination In other words, the duration of the action being constant, the optimum temperature of the ferment is independent of the concentration both of the substrate and of the enzyme The observation is suggestive: if true of one enzyme it may be true of all, and possibly becomes the enunciation of a general law. Herein, for the moment, lies its main interest.

* 'Phil. Trans.,' B, vol 203, pp. 351–371

† Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 87, p. 245 (1914).

‡ Gabriel Bertrand and A. Compton, 'Comptes Rendus,' vol. 157, p. 797 (1913).

§ For the variation of the optimum temperature of an enzyme with the duration of the enzyme action, see Gabriel Bertrand and A Compton, 'Comptes Rendus,' vol. 152, p. 1518 (1911); 'Ann. Inst. Past.,' vol. 26, p. 161 (1912).

In the present paper further experimental evidence for this hypothesis is given, in the case of another hydrolytic enzyme, the *maltase* of *Aspergillus oryzæ* (taka-diastase)

For the extract of *Aspergillus oryzæ* used, the Imperial Cancer Research Fund is indebted to Messrs. Parke, Davis and Co, who placed at my disposal one of their most active preparations This preparation, after being freed from insoluble constituents and purified by a technique to be detailed elsewhere, consists of a white powder, entirely soluble in water, whose activity in *maltase* is double that of the original preparation

The maltose used was Kahlbaum's It was purified by successive recrystallisations from water, the mother liquor impurities being removed after each recrystallisation by pressing the crystals in an hydraulic press between several layers of clean dry linen Eventually, after powdering in a mortar and drying for about a week *in vacuo* over sulphuric acid, a specimen of pure maltose, containing one molecule of water of crystallisation, was obtained It gave an optical activity $[\alpha]_D^{20} = +130 \cdot 4°$, and its reducing power, determined by Bertrand's method,* was as set out in Table I

Table I

Weight of maltose	Weight of copper
mgrm	mgrm
20 0	21 0
40 ·0	42 0
60 0	62 ·0
80 0	83 ·0
100 ·0	103 5

These numbers, allowing for the molecule of water of crystallisation present, correspond exactly with those given by Bertrand (*ibid.*)

That the optimum temperature of the ferment is independent of the concentration of the substrate is shown by the following experiments ·—Four series of eight clean Jena glass test-tubes were prepared containing respectively 360, 180, 90, and 60 mgrm of maltose dissolved in 4 cm³ of water which had been specially purified by redistillation under diminished pressure Then into each tube was introduced in portions of 1 cm³ a solution of the enzyme, prepared a half to one hour previously, containing 10 mgrm per cm³. The substrate concentrations in the four series of tubes are M/5, M/10, M/20, and M/30 The tubes, after being closed with clean sterile corks, were plunged into water-baths kept at known temperatures After

* 'Bull. Soc. Chim,' (3), vol. 35, p. 1285 (1906).

16 hours' incubation the tubes were withdrawn, the corks removed, and each rapidly washed with 1 cm^3 of water, the washings being carefully added to the contents of the corresponding tube The tubes were next heated for five minutes in boiling water to stop the enzyme action, they were then cooled, and the contents of each diluted to a known volume, such that 20 cm^3 of the diluted mixture corresponds to 36 mgrm of maltose The proportion of maltose hydrolysed was estimated by the increase of reducing power as determined by the method of Bertrand (*ibid.*) The numbers obtained are recorded in Table II

If the percentage of maltose hydrolysed be plotted against the mean temperature of the experiment these numbers give the series of curves represented by fig 1

Each curve shows a maximum at or about the same point, +47° Hence, under the conditions of the experiment, the optimum temperature of the ferment is constant, and independent of the variations in the concentration of the substrate.

That the optimum temperature is also independent of the concentration of the enzyme is shown by the following experiments —Four solutions of the enzyme were prepared containing 10, 30, 60 and 100 mgrm dissolved in 10 cm^3

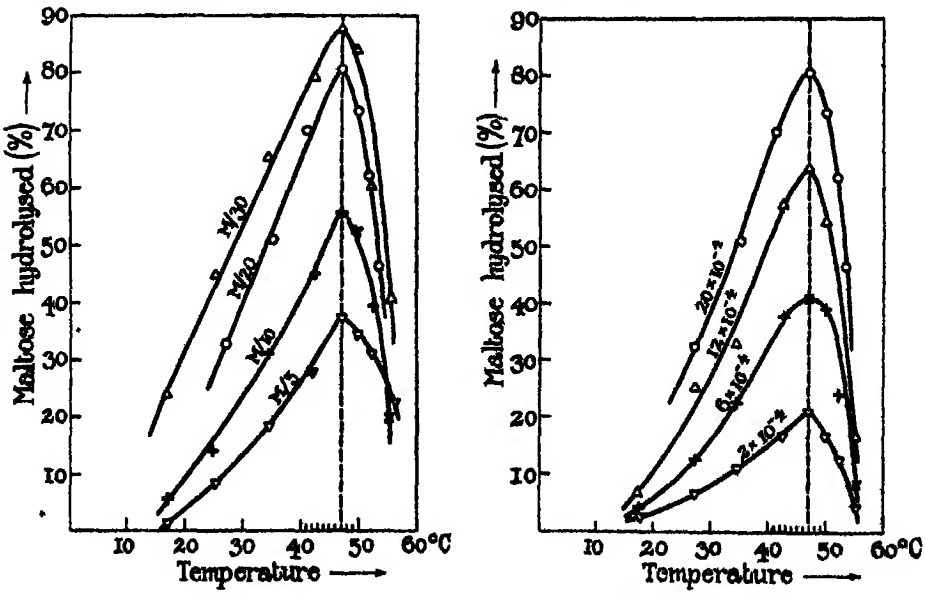


Fig 1 Fig 2.

Fig 1 —Substrate concentrations M/5 to M/30 Enzyme concentration 2×10^{-4} grm per cm^3

Fig. 2 —Substrate concentration M/20 Enzyme concentrations 2×10^{-4} to 20×10^{-4} grm per cm^3

Table II

Temperatures at the beginning and end of each experiment	Maltose hydrolysed per cent with the following substrate concentrations			
	M/5	M/10	M/20	M/30
17 0	1 9	—	—	24 0
17 2–17 0	—	5 9		
25·0	—	14 2		
25 8–25 5	8 6	—	—	45 0
27·0	—	—	32 8	
34 3–34 4	—	31 2		
34 5	18 3	—	—	65 2
35 0–35 1	—	—	50 9	
41·0	—	—	70 0	
42 4	28 2	—	—	78 5
42 5–42 4	—	45 0		
47·0	37 5	55·6	80 3	87 3
49 5–49·6	—	52 5		
49 5–50·0	34 3	—	—	83 8
50·0	—	—	73 4	
51 5–52 0	—	—	61·9	
52 2	31 2	—	—	60 3
52 2–52 4	—	39 1		
53 3–53 2	—	—	46 4	
54 5–55 2	—	19 7		
55 5–55·4	22 5	—	—	40 6

Table III

Temperatures at the beginning and end of each experiment	Maltose hydrolysed per cent with the following enzyme concentrations in grammes per cm³			
	2×10^{-4}	6×10^{-4}	12×10^{-4}	20×10^{-4}
17 3–17 5	—	4 5	7 2	
17 6	3 2			
27·0	—	—	—	32 8
27 3–27 5	7 2	12 8	25 4	
34 3	—	22 5	32 8	
34 5–34 4	11 4			
35·0–35·1	—	—	—	50 9
41·0	—	—	—	70 0
42 4–42 5	16 9	35 9	57 1	
47·0	21 1	40·6	63 5	80 3
49·8–49·9	—	39 1	54 1	
49·7–50·0	16·9			
50 0	—	—	—	73 4
51·5–52·0	—	—	—	61·9
52 1–52 2	—	24 0		
52·3–52 4	12 8			
53 3–53·2	—	—	—	46 4
55·2–55·0	4 5			
55·2	—	8·6	16 9	

of water, which, after standing from a half to one hour, were introduced in portions of 1 cm.³ into four series of test-tubes containing 90 mgrm of maltose dissolved in 4 cm.³ of water. The concentration of the substrate in this experiment is M/20, while the enzyme concentration varies between 2×10^{-4} and 20×10^{-4} grm per cm³ After 16 hours' incubation, the action was stopped, and the quantity of maltose hydrolysed in each tube was determined as before The numbers obtained are set out in Table III

On plotting the percentage of maltose hydrolysed against the mean temperature of the experiment the curves of fig 2 are obtained

Here, again, each curve shows a maximum in the same region of temperature, +47° Consequently, the optimum temperature of the enzyme is independent of the enzyme concentration.

Thus it is found, for the *maltase* of *Aspergillus oryzæ*—as for the *salicinase* of sweet almonds—that the optimum temperature of the ferment is independent alike of the concentration of the substrate and of the concentration of the enzyme

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A Theory of the Action of Rays on Growing Cells

By J Joly, Sc D., F.R S.

(Received May 28,—Read June 25, 1914)

The recent accessions to our knowledge of the nature of γ- and X-rays bring the treatment, by these rays, of malignant and morbid growths, into continuity with the treatment of lupus, etc, by the Finsen light or by other actinic radiation

The pathological effects of the shorter and more penetrating waves have been described by experienced observers as stimulative of the morbid growth when the administered radiation is feeble in intensity and as inhibitive of growth when the radiation is sufficiently intense Here there is plainly an effect produced by the short waves upon the growing cell, and the question arises if from this and allied observations we cannot gain some insight into the nature of the activity which characterises the malignant and morbid cell.

The well ascertained facts of photo-electricity show that, in all cases, the phenomena of direct light effects classed under that head are ascribable to the expulsion of electrons as a result of the vibratory energy communicated from the ether The loss of electrons is attended by ionisation of the atomic or molecular systems from which they are derived, the abstraction of the

negative charge leaving the positively electrified ion behind it This is the sufficient explanation of many phenomena collected under the name of photo-electricity. It has been ascertained that the velocity of the electron at the moment of its expulsion is the greater the shorter the wave-length of the radiation concerned The swift-moving β-rays represent the electronic discharge excited by γ- and X-radiations.

Some years ago I endeavoured to explain the nature of the events taking place in the photographic film in terms of photo-electric activity The theory has recently been republished and amplified by Mr H Stanly Allen in his book on photo-electricity

According to my view the latent image is formed of molecular systems which have been subjected to loss of electrons and which remain as ions positively charged in presence of these electrons, the nature of the medium being responsible for the maintenance of the static attraction between electron and ion In development these ions and electrons are discharged, and as a consequence of the chemical reaction thereby effected between the developer and the ionised photo-system the metallic atom is liberated, constituting the visible image The phenomenon of the reversal of the latent image under excessive light stimulus is well known On the theory this significant event is explained as the result of the increasing electrostatic stress attending over-exposure, whereby ultimately the resistance to recombination breaks down and the original molecular structure is restored The luminous stimulant will now begin to re-form the latent image A succession of such constructive and destructive effects is obviously possible according to the theory, and is, in fact, matter of observation. The theory can be shown to explain the facts respecting the different types of reversal as ascertained by R. W Wood. Classifying the modes of formation of the latent image as (1) by pressure, (2) by X-rays, (3) by light-shock (very brief flashes, as by light from an electric spark), (4) by lamp light, Wood found that the latent impression produced by any one of these can be reversed by subsequent exposure to any other following it on the list but not by any one preceding it. He found that Becquerel rays (γ-rays) behaved like X-rays For the manner in which Mr Allen applies the photo-electric theory to these observations I refer to his book

My object in referring to the photo-electric theory of photographic actions is to show that on the assumption that growth in the cell, generally, is attended and conditioned by ionic activity, there is sufficient resemblance between the effects of stimuli on the plate and on the cell to lead to the belief that there must be, physically, much in common between the actions in each case. *Prima facie* the formation of the normal latent image by

moderate light stimuli is parallel with the stimulation of growth by feeble X- or γ-radiation The photographic reversal by greatly increased illumination compares with the inhibition of growth by the heavy doses of γ-radiation now employed in the treatment of cancer.

The analogy when further pursued must take account of intrinsic differences prevailing in the two cases In the living cell there are continuous molecular movements and chemical interchanges accompanied by and attending the ionisation The static conditions reached in the latent image can only prevail for a brief period which terminates when the ions and electrons find fresh combinations The image-forming and reversing activities of the plate become respectively represented in the cell by the following events:—(*a*) Increased liberation of electrons (β-rays) and attendant formation of ions under the γ- or X-rays. This increases the metabolism, and, in the case of morbid growths, promotes the evil it is intended to cure (*b*) With increasing radiation sudden and excessive electrostatic stress (or over-ionisation) brings about immediate reversion to the original molecular state so that molecular changes and reactions are stopped and metabolism ceases The maintenance of this condition may lead to complete modification of the cell and ultimately to its absorption by the more stable normal cells which are not so readily influenced by the radiation An alternative view, less in line with the photographic analogy, is to suppose that, with increased density of electronic radiation emanating from all parts of the tissues, an ion freshly formed in the metabolic substance of the cell is almost instantly neutralised by a β-ray, so that the time required for the molecular movements attending metabolism is not given and growth ceases

In another particular we find the cell behaving in a similar manner to the photo-sensitive plate Physicians ascribe the origin of malignant growth in certain cases to continued local irritation. Here we have a parallel with the photographic plate, for the latent image, *i.e* the ionisation and electronisation of the film, may be obtained by various mechanical stimuli, such as pressure, friction, etc. The inhibition of the growth so produced in the tissues by γ-rays compares with the reversal of the pressure or friction marks of the film by light shock

The selective action exhibited by the morbid cell towards the radiation, so that these cells are soonest affected by the rays, is significant. The therapeutic value of the rays depends on this action To what may it be due?

Let us suppose the morbid cell characterised by less stable molecular systems than occur in the normal cell. In other words that the conditions obtaining in it are abnormally favourable to ionisation like a highly "ripened" photo-sensitive emulsion A feeble radiation will accelerate the activity of

the morbid cell and yet scarcely affect the normal cell, the latter corresponding to a "slow" photo-sensitive film Increased radiation which only attains the point of accelerating interchange in the normal cell may be attended by a sufficiently dense β-radiation to inhibit the metabolism in the morbid cell in the manner already suggested. In other words—to revert to the analogy with the photo-sensitive salt—the amount of ionic and electronic stimulus which builds the latent image in the "fast" film is insufficient to affect the "slow" film and as the stimulus is increased the latent image of the first suffers reversal at a point which builds up the latent image in the second. This appears to be just what is observed in the case of radiation treatment, the success of the method depending upon a lag in the effects arising in the normal tissues, as compared with those arising in the morbid tissues

It may also be urged for the present view that if the effects of γ-rays on the growth of the cell are not of a photo-electric character, and so productive of ionisation, we must recognise in them some quite new reaction between matter and light This seems a needless course when there does not appear to be any *a priori* objection to urge against the unification of our views respecting the photo-stimulation of the sensitive salt and the effects of γ-rays on the molecular systems existing in the cell

Assuming a real basis for the approximation of the two processes, the question as to how the peculiar constitution of the morbid cell may arise deserves more careful consideration than I am competent to give to it Upon the photographic analogy we might reason thus —If, in the life of the cell, ions are naturally always being formed, the absence of a "restrainer" might lead to morbid ionisation, or, again, the presence of a "sensitiser"—the former to limit the ionising activity either physically by its inert properties, or chemically, the latter to accelerate it by removing the products of reaction as fast as they are formed. Dr. Lazarus-Barlow, however, has found notable and excessive quantities of radium in certain tumours. If this was general to all spontaneously arising cancers we might find here a sufficient cause of excessive ionisation. In this connection it is perhaps significant that the study of the distribution of cancer has been found to follow in a notable way the nature of the soil constituents of the district. Thus it is stated that cases of cancer are more frequent in clay-covered areas than in calcareous regions. Now calcareous rocks are almost without radioactive constituents, whether of the uranium-radium series or of the thorium series. The amounts of emanation continually being exhaled from such soils must be very different. It would be interesting to directly examine the several districts for soil-emanation.

Again, the well-known prevalence of cancer among chimney sweeps may be associated with the fact that charcoal and other forms of carbon, which must enter largely into the composition of soot, absorb radium emanation readily from the atmosphere It is improbable that sweep-cancer is ascribable to skin irritation only, seeing that many other occupations (*e g* stone working, cement making) are exposed to even greater risks from that source.

On the theory that the cancer cell is the seat of excessive ionisation, we may ask if it is possible to control its activity The latent image, although not possessed of the progressive fluxional characters of the cell, is, potentially, such an active configuration. It may be destroyed (*a*) By such a light stimulus as will bring about reversal The radioactive treatment of cancer is—on the present theory—an application of this fact. (*b*) By development, *i e* by such a chemical treatment as serves to discharge the ionised systems The finding of a reagent which would act similarly on the morbid cell is, perhaps, not impossible. It would have to act selectively towards the less stable cell and must itself be ionised or become so in process of application It would discharge the function of diverting the ionising activity to the formation of inert and harmless products

In a sense we may regard development as continually progressing in the organic system, much as if a light-sensitive salt were maintained submerged in a developer while exposed to light. From this point of view it might be better to seek the intervention of a "restrainer" which would either retard molecular motions of diffusion, etc, in a mechanical way, *i e* by viscosity—as many restrainers are believed to do—or by chemically altering the nature of such conditions as result in growth and metabolism If such remedies could be applied through the circulatory system, so as to reach metastases, depressing and lowering the abnormal ionic activity or directing its results into harmless channels, curative treatment might be attainable.

The theory here suggested for the processes going on in a cancer cell is a physical one, or, it may be said, takes account of the physical aspect primarily, and would involve the probability of successful treatment by experiments directed along physical and chemical lines But it is not suggested that the origin of, or predisposition towards, abnormal ionic activity may not be founded in biologic causes Nor does it enter into, or take account of, the probably extremely complex nature of the events progressing within the cell as leading to, or resulting from, the physical actions referred to in the theory.

The Influence of Timbre and Loudness on the Localisation of Sounds.

By Charles S Myers

(Communicated by Prof. C S Sherrington, F.R S Received June 3,—Read June 25, 1914)

1 *Introductory*

In analysing the factors determining our localisation of sounds, it will be found convenient to distinguish "laterality" from "incidence" By the laterality of a sound I mean its apparent position in relation to the median vertical front-to-back, or "sagittal," plane; thus, a sound may give the impression of rightward or leftward laterality, or it may appear to have zero laterality—that is to say, its position may seem to be in the median plane By the incidence of a sound I mean its apparent position in relation to the horizontal "interaural" or "coronal" line, thus, a sound may give the impression of more or less upward, downward, forward, or backward incidence, or it may appear to be directly sideward, neither above nor below, neither in front of nor behind, the interaural line—when the incidence is of zero value

I consider it important to distinguish at the outset these two elements in localisation, since they are dependent on very different factors In normal subjects, that is to say, in persons who have normal binaural hearing, the one certain and obvious determinant of laterality consists in binaural differences of intensity. A sound is localised on the side of that ear which receives the stronger stimulus, it is localised in the middle line, midway between the two ears, when they are equally stimulated by the sound *

But such binaural differences of intensity must clearly fail as a basis of our determination of incidence Whether a median sound lies immediately in front of or behind us, or whether it is placed immediately above or below our

* Another determinant of laterality, binaural differences of wave phase, was suggested in 1907 by Lord Rayleigh ('Phil. Mag,' vol. 13, pp 214–231, 316–319), but, taking into consideration the physiological fact that, owing to the bone conduction of sound across the skull, it is impossible to stimulate one ear without stimulating the other, I have indicated, in collaboration with H A Wilson ['Roy. Soc. Proc.,' A, vol. 80, pp 260–266, 'Brit. Journ. Psychol.,' vol. 2, pp. 363–385 (1908)], how the effects of binaural phase differences are ultimately explicable in terms of the differences in binaural intensity to which they may be supposed to give rise Lord Rayleigh has since ['Roy Soc. Proc.,' A, vol 83, pp. 61–64 (1909)], allowed that "for the moment the choice between the competing views [as to the manner in which phase differences at the two ears produce their effect] is likely to depend upon preconceptions as to the manner in which the nerves act"

head, it must stimulate the two ears with the same intensity. It is just under these conditions that our localisation becomes erratic. As is well known, a sound coming from in front is apt to be localised behind, and *vice versâ* So, too, in regard to sounds placed before and behind the ear: a sound produced midway between the front and the side of one ear is often localised midway between the back and the side of that ear, and so on

It has been found that, although extremely erratic, our determination of the incidence of a sound is capable of enormous improvement by practice, and, seeing that our accuracy is greater with sounds richest in overtones,* it has been supposed that our awareness of incidence is dependent on the variations of timbre which occur with variations in the angle at which the sound waves impinge on the auricle

Now, if it be true that variations in timbre are responsible for our determination of the incidence of a sound, it should be possible to put this assumption directly to the test by experimentally varying the timbre of a given sound while its position is kept constant, and by observing what changes, if any, in its apparent position are produced thereby Such has been the main purpose of the experiments described in this paper, and, as will be seen, they afford a striking proof of the correctness of the assumption

Two other possible factors affecting sound localisation have yet to be mentioned. It has long been recognised that sounds coming from in front of the subject's auricle are better heard than those coming from behind. The auricle is so inclined and is so formed as to "catch" forward sounds better than rear ones † Such variations in loudness, according to the relative positions of the sound and of the ear, may conceivably help in determining the incidence of the sound. The other possible factor, assisting the determination of laterality and incidence, consists in the tactual sensations which vibrations of sound may conceivably evoke by their contact with the auricle, the external meatus, or the tympanic membrane. The experiments described in this paper also afford some estimate of the value to be attached to these two factors.

II *Experimental Methods.*

The experiments were conducted in a sound-proof room (R, fig. 1), the walls and ceiling of which, composed of stone, peat-moss, and cork

* Angell and Fite, 'University of Chicago Decennial Publications,' vol. 3, part 2 (1902)

† How the ear "catches" sounds is quite unknown. The old explanation of reflection of the sounds from the concha to the tragus, and thence into the meatus, is untenable in view of the disproportion between the size of the ear and the length of the sound waves.

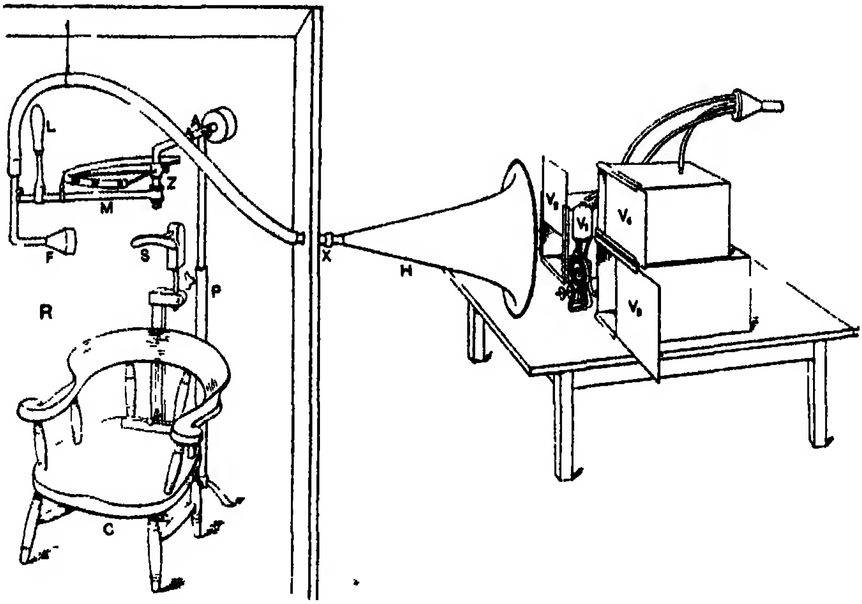

FIG 1

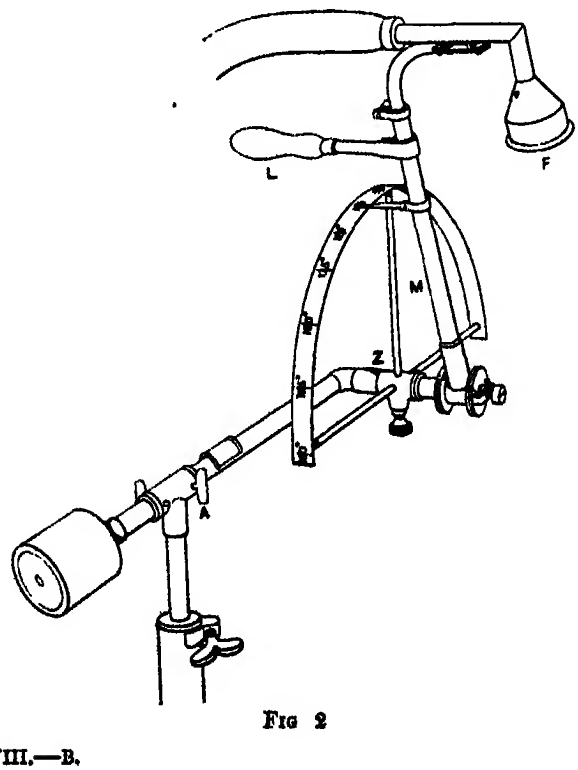

FIG 2

composition, were covered on their inner surface with a thick layer of horsehair The floor, also isolated from the rest of the building, was similarly covered. By this means the reflection of sounds from the walls and floor was reduced to a minimum The subject sat blindfold in the centre of the room. A sound perimeter (P, *cf* also fig 2) was constructed for these experiments. It consisted essentially in an arm M, silently rotatable about Z by means of the handle L, and carrying a funnel-shaped mouth F, which was connected by a flexible pipe with the sound-producing apparatus in a neighbouring room. The centre about which the arm of the perimeter rotated was always placed over the point midway between the two ear-holes. The perimeter could be turned round the axis A so as to give sounds in the vertical as well as in the horizontal plane

With the perimeter arranged as in fig 2 the sound could be presented at any point in the median vertical (sagittal) plane, *i e.* directly in front of (= 0°), above (= 90° v) or behind (= 180°) the subject, or at any intermediate point. The sound could also be presented, as in the arrangement of the perimeter shown in fig 1, at any point in the horizontal plane at the level of the ears, *i e* directly in front of (= 0°) or behind (= 180°) the subject, or exactly opposite the right or left ear (= 90° h or 270° h.) or at any intermediate point

The pipe connecting the funnel with the sound-producing apparatus was enveloped with layers of cotton wool and bandages, and passed through a tube in the wall of the sound-proof room to a very wide-mouthed horn H, such as is used in connection with the phonograph when heard in large halls Before this horn were arranged four of Stern's tone-variators, V_1, V_2, V_3, V_4, blown by wind from a hydraulically worked organ bellows. These tone-variators, one of which, V_1, was unenclosed, produce relatively pure (overtone-free) tones, they are essentially bottle whistles, each consisting of a mouthpiece fixed over a metal air-containing cylinder. The pitch of the note depends on the height and circumference of the cylinder, the base of each cylinder being movable so as to adjust the pitch accurately. The first or largest tone-variator was arranged to emit a (fundamental) tone of 215 vibrations per second. The second variator gave the first overtone, the third gave the second, and the fourth gave the third overtone, of this fundamental, *i.e.* they emitted tones of 430, 645, and 860 vibrations per second respectively. In order to reduce and to vary at will the loudness of these overtones, the three corresponding variators, V_2, V_3, V_4, were each enclosed in a wooden box, open at one end. The open end of each box could be more or less completely closed by means of an adjustable slide, thus allowing the intensity of the overtones (and hence the timbre of the

total sound) to be experimentally varied.* Three positions of the slides were adopted position B, the middle or "normal" position of the slides, which was used for practising the subjects in sound localisation, position A, in which the slides nearly closed the open ends of the boxes, and position C, in which the slides were drawn well out so as to produce relatively loud overtones

The loudness of the whole sound (fundamental and overtones) was varied by moving the horn nearer or farther from its middle or "normal" position The subjects were practised in sound localisation with the horn at its "normal" or B position Subsequently, the loudness of the sound was decreased by moving the horn farther from the tone-variators (the A position of the horn) or increased by moving the horn nearer to the tone-variators (its C position)

The use of the tone-variators and of varying positions of the slides or horn, just described, necessitated the presence of an assistant in the room in which the sounds were produced. Communication between him and the experimenter, who sat with the subject in the sound-proof room, was effected by means of loud-speaking telephones and an electric bell, so that by pre-arranged signs the assistant might give the sounds at the desired moment, and vary their timbre or loudness in the desired order

Experiments were also carried out in which the sound was produced by the experimenter within the sound-proof room by means of a telephone buzzer or an electric bell placed at the position of the funnel F, at the free end of the rotating arm of the perimeter

Except when otherwise stated, the mode of procedure was as follows The subject, seated in the chair C in the sound-proof room, was blindfolded, and a head rest was adjusted to the back of his head in order to prevent, so far as possible, any movement. The perimeter was then arranged so as to allow of sounds being given in one or other of the two planes (vertical or horizontal), and the slides and horn were set at their respective B positions Several sittings were given by each subject for practice in localisation, and later the positions of the slides and horn were irregularly varied for the study of the effects of variations in the timbre and loudness of the sound in one of the two planes, before similar experiments were made in the other plane. The production of the sound which the subject was required to

* In a number of preliminary experiments, I employed resonators at variable distances from the variators, but the tones conducted from the resonators by narrow rubber tubes to the sound-proof room were too weak for my purpose. I also tried a loud-speaking telephone for conducting the tones, but, owing to the unsatisfactory timbre and inconstancy of the resulting sound, I had to abandon this method likewise.

localise was preceded by a warning "Now", and the sound was allowed to last for about two seconds. Immediately after each sound was given the subject was required to indicate its supposed position. In the early stages of practice the sounds were given at any position within the half circle (from 0° to 180°) of the plane concerned, and the subject's forefinger was armed with a large graduated quadrant carrying a freely movable index, so that when he pointed to the apparent direction of the sound the index registered the angle at which the sound appeared to be placed. In the later experiments, when only three positions of the sound in any one plane were employed, and the subject was either being instructed in correct localisation or (still later) being tested for the effects of variations in timbre and loudness, he learned to return his answers orally in terms of the angle from which the sound appeared to come.

Eleven subjects were investigated, seven male and four female, all under 40 years of age. Each sitting lasted about 40 minutes, and each subject gave from three to six sittings, making from 200 to 400 judgments of localisation.

III. *Experimental Results.*

1. *Localisation before Practice.*—

(a) *For Sounds in the Median Vertical Sagittal Plane.*—Without practice the complex sound from the variators proved extraordinarily difficult to localise. Whatever the actual position of the sound, some subjects localised it in front, others localised it behind, others were unable to give any consistent localisation. As one subject remarked, "I could put it anywhere; I seem to think out where it might be and then it seems to be there." Another subject reported, "When you tell me where it comes from, I see it can do so, and can place it there."

When the variators were replaced by a telephone buzzer before the horn, no appreciable difference in the certainty or accuracy of localisation was observable.

It was always difficult to arrange the apparatus so that the sound appeared exactly in the middle line. At first the difficulty was traced to a slight leakage of the sound through the flexible tube which conducted the sound from the inlet pipe in the wall to the funnel-shaped opening borne on the perimeter. But even when this difficulty was surmounted, the slightest error in the position of the perimeter in regard to the sagittal line of the head immediately occasioned lateral (right or left), instead of purely median, localisations.

Wondering whether any possibly still remaining leakage of sound during transmission could be responsible for the extreme difficulty and inaccuracy

of localisation of the variator sounds, I replaced the funnel-shaped mouth first by an electric bell, later by a telephone buzzer, in the expectation of obtaining more accurate and certain localisation when the sound was generated on the perimeter instead of being conducted to it from the room outside The same diverse and erratic localisations were maintained Some subjects never localised the sound behind 90° v. if it was placed at 180°, others never localised in front of 90° v a sound given at 0° Here, for example, are the records of two subjects, J. and Ss, for sounds of the buzzer (affixed to the perimeter) at 0°, 90° v and 180°.—

	J						Ss.		
0° ...	0°	0°	0°	0°	0°	0°	180°	180°	180°
90 v .	90	120	90	90	90	90	135	135	135
180	90	90	90	90	90	90	130	200	200

Before I had obtained evidence of these striking individual differences in localisation, I wondered whether inequalities in the reflexion of the sound from the four walls of the room could be responsible for the gross errors met with. Accordingly, on several occasions, I reversed the position of the subject, testing him now with his face, now with his back to a given wall With these changes one subject was tested with the variator sounds, two subjects with the buzzer sound conducted from the room outside, and one subject with the bell ringing on the perimeter But in no case was any change in localisation relatively to the subject observable. If he had localised all sounds to his rear in one position, he continued to localise all sounds to his rear in the reversed position, and so on.

This result is striking evidence of the influence of natural tendencies and prejudices on the part of the subject in his localisation of sounds placed in the median sagittal plane The influence of expectation was also clearly demonstrable by directing the subject's attention forwards or backwards at the moment of the production of the sound, whereupon the apparent position of the sound was generally changed in the sense of such direction of the attention.*

(b) *For Sounds in the Horizontal Plane.*—But if the ability to localise fore and aft sounds in the median vertical sagittal plane is so defective, we should not expect to be better able to localise fore and aft sounds placed along the horizontal plane; for both kinds of localisation are instances of what I

* The following conversation between subject (S.) and experimenter (E) will serve to illustrate this feature :—S. "I expected a sound behind and I got it [sound given at 0°] there." E. "Now try to imagine it in front" [sound at 0° repeated]. S "Yes, I certainly get it there, too." E. "Now try to imagine this sound [at 180°] behind." S "Yes, certainly it is there, but when I change my idea to its being in front, I get it there too'

have termed incidence. Laterality can only concern whether the sound is placed to the right or left of the subject or somewhere in the median line, and, as I have already said, errors in laterality were never found in these experiments, provided that the auditory acuity of the subject's two ears was normal and that the position of the sounds relatively to the two ears was such as to produce the required binaural difference (or equality) of loudness. On the other hand, whatever factors are responsible for the determination incidence should hold for the horizontal, as well as for the median sagittal, plane

Experiments carried out on five subjects with variator sounds given in the horizontal plane reveal just the same inaccuracies as have been described for the median sagittal plane The first of these subjects localised all sounds—whether fore (45° h), side (90° h), or aft (135° h)—behind his ear, the second localised them all in front of his ear, the third localised fore and aft sounds in front of his ear, while the fourth and fifth subjects gave too variable a localisation to allow of any more general statement than that they showed total inability to distinguish fore, side, and aft sounds from one another

Two questions naturally arise —How is it that previous observers, while recognising a liability to err in the localisation of such sounds, have not laid stress on the initial grossness of the errors of localisation revealed under the conditions of these experiments? How is it that these errors do not play an equally prominent part in our everyday life? We are all aware of occasional errors in fore and aft localisation, but it is relatively seldom that they are brought to our notice

Now, one important factor consists in familiarity with the sound. As we shall see, with practice every subject learned to localise correctly Another important factor employable in everyday life, but eliminated to a very large extent in these experiments through the use of a head rest, consists in head movement On several occasions in the course of these experiments I expressly instructed my subjects to move the head while they were listening to the sound, whereupon their errors in localisation were immediately and often quite accurately corrected

In some experiments, moreover, performed in the open air, in which I acted as subject, where the vowel E was spoken by an assistant and his position had to be ascertained, I localised both fore and aft positions forward, but when the experiments were repeated with a small head movement carried out during the production of the sound, I at once changed the localisation of the rearward sounds from fore to aft

Obviously, by turning the head, the sound is alterable in intensity; for,

as I have already mentioned, the position of the auricle is adapted for "catching" sounds coming from in front (and in consequence of which our auditory acuity is keener for forward than for rearward sounds) But turning the head alters, too, the timbre of the sounds, a forward sound appears to the ear not only louder than, but also of a different timbre from, the sound placed to the rear *

It is noteworthy, however, that, whereas change in the position of the head while the sound was being heard was remarkably effective in correcting errors of localisation, change in the position of the sound while the head was at rest proved of little or no advantage for such correction It generally resulted in an interpretation of increased or diminished loudness, or of increased or diminished distance of the sound, less frequently, a movement of the sound was detected, but the direction of the movement was not always correctly given, and the initial error in localisation failed to be corrected by the detected movement of the sound This difference in effect between what may be conveniently termed "active" and "passive" change in the position of the sound is of considerable interest in relation to the associated function of the semicircular canals and (in animals) of the movements of the auricle

Two other factors which are conceivably of importance in determining the incidence of sounds, but which were almost wholly eliminated in these experiments, may be briefly mentioned Of these the influence of expectation has been already alluded to on p 273, and was almost always successfully ruled out by the noiseless movement of the perimeter On several occasions I expressly asked the subjects if they had any notion of where the sound was coming from, and they generally replied that they had no idea In everyday life, however, and, perhaps, in many of the experiments otherwise conducted, various cues may determine a favourable attitude of expectation in the subject. The remaining factor, the effect of sound reflections from the ceiling, walls, and floor, was prevented by the peculiar construction of the sound-proof room (pp 268–270). But in everyday life and after brief practice in experiments, conducted under ordinary conditions, there are indications that such reflections are taken into account and thus assist in determining the incidence of the sound.†

* It is practically impossible to increase the loudness of a sound (i e. the intensity of the fundamental and its overtones) without altering its timbre (the relative intensity of the fundamental and its overtones) Even if this could be physically realised, the varying position of the peculiarly formed auricle relatively to the sound may be expected to influence the ease with which it takes up the different overtones contained in the sound.

† Since writing this, I have examined two subjects, first in an ordinary room, and later (after a rest) in the sound-proof room, using the perimeter with an attached

2. *Localisation during Practice*—

(a) *For Sounds in the Median Vertical Sagittal Plane.*—The practice experiments were carried out during several sittings, the number (*cf* p. 272) depending on the rapidity of improvement in the subject's accuracy of localisation. The subjects were now told when they were right or wrong, and only three positions of the sound were given—at 0° directly in front, at 90° v directly above, and at 180° directly behind an imaginary line between the two ears.

The final result was always to establish absolute accuracy in the localisation of the sounds. But the three positions were not learned with equal ease, consequently the total number of right answers varied with the position of the sounds, the figures during relatively late stages of practice with the variator sounds being—

For	0° .	80 per cent	of answers correct.
„	90 v . .	72 „	„
„	180	67 „	„

The criteria apparently employed during the subject's practice, in order to distinguish these three positions, were (i) so-called "tactual" experiences; (ii) right or left laterality, (iii) difference in timbre, loudness, or nearness. Of (i) further mention will be made later (pp. 280–283). Reliance on (ii) was only possible when the sound was not accurately produced in the middle line, if, for example, the rotating arm swung a little obliquely from before backwards, the subject came to realise that when the sound was heard (say) to his left it was placed (say) behind him, whereas when it appeared to his right it lay to his front. In regard to (iii) various subjects stated that at 0° the second was "fuller," "more voluminous," or "more open," while at 90° v. it was "duller," "drearier," "more drony," or "more booming," and at 180° it sounded "rather like an echo," "faint," "lacking in assurance," "fuller than at 90° v., though very like it," yet "duller and more distant than 0°."

Similar results were obtained during practice when the telephone buzzer took the place of the variators before the horn.

(b) *For Sounds in the Horizontal Plane.*—In these experiments only three subjects received practice for sounds placed at 45° h., 90° h, and 135° h., but the results were precisely similar to those obtained for the sounds in the vertical plane. Two subjects thought that at 90° h. they could distinguish

electric bell and buzzer Despite the practice gained in the ordinary room, their errors increased by about 50 per cent. in the sound-proof room, showing clearly the influence of the strange environment. Over 300 judgments were obtained.

(i) "tactual" sensations. Of course in the positions used in the horizontal plane (ii) laterality could afford no clue as to the fore or aft localisation of the sound. The remaining factor (iii), differences in timbre, loudness, and nearness, proved the most important criterion in learning to localise the sounds correctly One of the subjects complained of special difficulty in distinguishing sounds at 90° h. and at 45° h., another of special difficulty in distinguishing sounds at 90° h and at 135° h. At 135° h the sound seemed to two subjects "more remote and weaker," "less clear and less distinct," than at 45° h. or 90° h., the third subject, however, observed that at 135° h. it was "nearer and more rounded," "not so veiled," as at 45° h.

3. *Experimental Variations in the Timbre and Loudness of the Sounds*—

(a) *For Sounds in the Median Vertical Sagittal Plane*.—In the case of two subjects, after being thoroughly practised in the correct localisation of sounds given at 0°, 90° v, and 180°, instructive results were obtained by experimentally varying (i) the timbre and (ii) the loudness of the variator sounds. In both subjects variations in timbre (produced by varying the position of the slides) yielded less striking errors of localisation than variations in loudness (produced by varying the position of the horn) Thus in one subject, who had just given 17 of 18 answers correctly for the normal or B position of the slides and horn, variations in the position of the slides produced one doubtful and one ambiguous* answer in 11, while variations in the position of the horn gave one wrong and four doubtful or ambiguous answers in nine. On another occasion the same subject, after giving two doubtful or ambiguous answers in 18 for the normal position of the slides and horn, gave 10 wrong answers and one doubtful answer in 18 when the position of the horn was varied. The disturbing uncertainty thus produced was in some degree carried over to the subsequent experiments immediately carried out with variations in the position of the slides, when three wrong and two doubtful or ambiguous answers in 13 were returned.

The second subject, who was examined only for the positions 0° and 90° v., gave five wrong and two doubtful or ambiguous answers in 27 for the normal or B position of the slides and horn, followed by three wrong and two doubtful or ambiguous answers in 18 when the position of the slides was varied On another occasion, when the same subject had just given 12 consecutive right answers for the normal or B position of the slides and horn, six wrong answers in 21 were obtained by varying the position of the horn. In both subjects it was found that, whereas the sounds at 0° suffered least,

* An answer is "doubtful" when the subject is obviously uncertain; it is "ambiguous" when the subject ascribes to the sound alternative positions, of which one is correct.

those at 90° v suffered most in the accuracy with which they were localised under the above conditions.

These results may be tabulated thus, the figures showing the percentages of error, doubtful or ambiguous answers being counted as half errors, wrong answers as whole errors, and the two vertical columns for each subject representing the results respectively obtained from the two sittings at which each was examined:—

	Subject I		Subject II.	
Horn and slides in B position .	6	6	22	0
Horn in B position, slides in varied position	14	31	22	—
Slides in B position, horn in varied position	33	58	—	29

Six times, the near or C position of the horn caused a sound at 90° v to be located at 0°, and on two occasions, one at 180° to be located at 90° v Three times, immediately following a sound given with the open or C position of the slides, a sound given at 0° with the B position of the slides was located at 90° v., and on two occasions, immediately following a sound given with the B position of the slides, a sound given at 90° v with the C position of the slides was located at 0°.

The following answers illustrate the difficulties in which the subjects found themselves, and indicate the bases of their judgments of localisation —

Sound given		Subject's reply
	°	
Slides C	90 v	0° "Because it was so full; yet it seemed perfectly vertical and hit me on top of the head"
„ C	90 v	0° or 90° v "It seemed loud, hence front; yet far away, hence top"
„ A	0	? 90° v, hesitation "Because, though not so weak as a top sound, yet it does not seem so direct as a front one."
„ A	0	90° v. "Because it is so faint"
„ A	0	? 0° "It is rather weak, though, for a front sound"
Horn C	90 v	? 0° "It has the character of a front sound in coming from a distance, but it's so drony and dreary"
„ C	90 v	0°. "Its character resembled the previous sound [B, 90° v], yet it came from so short a distance as to seem front"
„ A	0	90° v "It's drony, yet it's rather too loud for top"

(b) *For Sounds in the Horizontal Plane*—The influence of changing the timbre and loudness of the sounds on their localisation is not less marked for sounds in the horizontal plane, although certain differences are to be noted. In the following record of one of my subjects the first two columns give the actual, and the third column gives the apparent positions of the sound, the observations of the subject being given in footnotes —

Sound given		Subject's reply	Sound given.		Subject's reply
	°	°		°	°
Slides A	135 h	45 h	Horn B	90 h	? 90 h
„ B	135 h	135 h	Slides A	135 h	45 h †
„ B	45 h	45 h	„ B	90 h	90 h
„ B	90 h.	90 h	„ C	135 h	135 h
„ B	135 h	135 h	„ B	45 h	45 h
„ B	45 h	45 h	Horn A	90 h	90 h
„ C	45 h.	135 h	„ C	90 h	90 h
„ B	135 h	135 h	„ B	45 h	45 h
„ B	90 h	? 90 h	Slides A	45 h	45 h
„ B	135 h	135 h	„ B	45 h	45 h or 90 h
Horn A	90 h	? *	Horn A	90 h	45 h or 20 h
„ B	45 h	45 h	„ C	45 h	45 h
„ B	135 h	135 h	„ B	135 h	135 h
„ B	135 h	135 h	Slides A	90 h	45 h
„ B	90 h	90 h	„ B	90 h	45 h or 90 h
Slides C	45 h	45 h	„ C	45 h	45 h

* "It has the quality of 45° h, but it is not so far back, I think, nor so distant as 90°"
† "That's the 45° h all right!"

That is to say, for 19 estimations in the normal or B position of the horn and slides, only four doubtful or ambiguous answers occurred (11 per cent. of errors), whereas for six estimations in the A or C positions of the horn there were two such answers (17 per cent of errors), and for seven estimations in the A or C position of the slides there were four wrong answers (57 per cent. of errors) Thus the effect of varying the loudness of the sound was to reduce the certainty of this subject's answers, while the effect of varying the timbre of the sound was to change the apparent position of the sound.

It will be noticed that whereas changing the positions of the horn produced greater confusion in the vertical plane, changing the position of the slides produced greater confusion in the horizontal plane We might be inclined to conclude from this that localisation is based on differences in loudness for sounds in the vertical plane, and on differences in timbre for sounds in the horizontal plane. But we have to remember that changes in the position of the horn must have affected not only the loudness but also, though much less markedly, the timbre of the sound, and that changes in the position of the slides must have affected not only the timbre but also, though much less markedly, the loudness of the sound

We have also to bear in mind that in the vertical plane we were dealing with sounds placed at forward (0°), topward (90° v), and backward (180°) positions, while in the horizontal plane, the sounds were given half-forward (45° h.), to the side (90° h.), and half-backward (135° h).

We may, I think, legitimately conclude that for sounds given at 0°, 90° v, and 180°, our localisation is based principally upon differences in loudness,

whereas for sounds given at 45°, 90°, and 135° in the horizontal plane our localisation is based principally upon differences in timbre; "principally" because changes in the position of the horn must have affected not only the loudness but also, though much less markedly, the timbre of the sound, and because changes in the positions of the slides must have affected not only the timbre but also, though much less markedly, the loudness of the sound.

This conclusion is in harmony with other considerations There are enormous differences between sounds at 0°, 90° v, and 180°, as regards the favourableness of their position for being "caught up" by the pinna. The pinna catches sounds coming from the front better than it catches those coming from the rear, as is well known, auditory acuity is keener forwards than behind. It is hence not surprising that we learn to distinguish fore, aft, and top sounds principally by differences in loudness On the other hand, sounds given at 45°, 90°, and 135° in the horizontal plane must differ little in loudness, the difference between the extreme positions, 45° and 135°, is much less than that between positions 0° and 180°; 45° and 135° are almost, although not quite, equally favourable positions for the sound to be caught up by the pinna, and indeed 135° is the angle most suitable for the direct entry of the sound into the meatus

4. *The Rôle of Tactual Sensibility in Auditory Localisation*—

These experiments appear to prove conclusively not only that variations in timbre and loudness are responsible for our determination of the incidence of sounds but also that cutaneous sensibility can play no part whatever in sound localisation That cutaneous sensations can play no part so far as concerns laterality is shown by the well-known fact that whereas we are able correctly to localise two simultaneous tones of clearly different pitch, placed one on each side of our head, whatever be their relative loudness, our localisation of two tones thus placed, when they are of identical pitch, depends upon their relative loudness, if the two tones are equally loud, the sound is localised in the median plane, as soon as they become of unequal loudness, the sound is immediately localised in that ear which receives the stronger stimulus.*

Now, if the sounds falling on each ear gave rise to tactual sensations, there can be no reason why, whatever their pitch and relative loudness, two such tones should not be correctly localised, one on one side of the head, the other on the other. On the other hand, it is quite clear that sound localisation rests on an auditory not on a tactual, sensory basis, since when the tones are of identical pitch only a single sound is heard and its localisation is

* I omit for simplicity's sake the consideration of phase difference here (see, however, footnote to p. 267)

accordingly ascribed to a single position, median or lateral, instead of to two lateral positions.

Further, when the tones are of different pitch, it is impossible to see how tactual sensibility can be the basis of their separate localisation For suppose that one pinna, meatus or drum receives a series of tactual stimuli from the one tone, and that the opposite pinna, meatus or drum receives another series from the other, it is inconceivable how the subject can refer these two series of tactual stimuli to their respective tones, how can he decide which tone to allot to which ear merely on the basis of his tactual sensations? Again, suppose that a subject has become absolutely deaf in both ears, why on the hypothesis of tactual localisation should he not still be able on request to localise successfully a sound stimulus though unable to hear it as sound? Yet this is inconceivable save in the case of the very lowest tones, the stimuli of which evoke tactual as well as (indeed ultimately in place of) auditory sensations Moreover, the unimportance of the tympanic membrane in sound localisation is shown by the preservation of localisation in cases where the membrane has been removed through disease, and in cases of tinnitus aurium where the sensations although localised arise subjectively, within the inner ear

That tactual stimuli received by the pinna play a part in the localisation of sounds in the median sagittal plane is rendered highly improbable by *a priori* considerations. The following experiment, moreover, appears decisive After preliminary practice, I acquired correct localisation of sounds in this plane, whereupon I placed a short piece of narrow rubber tubing in each ear, the result of which was to make an obvious change in the loudness and timbre of the sounds heard. Now if the pinna had been responsible for the previously correct localisation, no change should have resulted from the insertion of the rubber tubes into the two meatus But in point of fact, I was quite unable to localise the sounds correctly, and had to start afresh re-learning them. There was no doubt in my mind that I had based my previously correct localisations on changes in the relative loudness and timbre of the sounds dependent on their position in regard to the ears. The results of this experiment confirm those already described in this paper showing the definite changes in localisation produced by definite changes in the loudness and timbre of the sounds.

Nevertheless, the belief that auditory localisation is, at bottom, of tactual origin dies hard. Started by Weber* and perpetuated by Wundt† and others, the tactual hypothesis has been recently invoked by Hocart and McDougall‡

* 'Ber. d. Kgl. Sächs. Ges. d. Wiss.,' 1848, p 237; 1851, p. 29

† 'Grundzüge der Physiologischen Psychologie,' 5th ed., vol. 4, p. 487

‡ 'Brit Journ. Psychol.,' vol. 2, pp. 386–405 (1908).

to account for their experimental results. In my own experiments, were I to trust the introspective data of several of my subjects, additional evidence could be supplied in favour of this view.

Thus for sounds given in the median sagittal plane, one subject in her early stages of practice described those at 0° as follows: "It hit my head just above the forehead," "it hit me just above the forehead," "it hit me just in front of the top of my head," while the 90° v. sounds seemed to have "a more vertical feeling," "a straight-downward feeling" Now to this subject all sounds (in the median sagittal plane) at first appeared to come from the ceiling cornice in front of and above her Most descended and hit her forehead and vertex, while a few others remained there Sounds at 180° were accordingly described thus · "It remained on the ceiling, but pointed to the forehead", "it was located in front of and above me at the ceiling cornice, but it struck me at once on the vertex"; "it seemed a little lower than the rest, but it hit me in the middle of the forehead." Another subject, who at first ascribed a backward position for all sounds in the median sagittal plane, described the 0° and 90° v. sounds as hitting him at the occiput, and the 180° sounds as hitting him at the nape of the neck Yet another subject reported on 90° v—"that reached my eye instead of my ear."

In the case of sounds in the horizontal plane similar examples may be quoted. One subject, who at the start ascribed a position of 45° h. to sounds given at 45° h and at 135° h. and a position of 22° h. or 45° h. to sounds given at 90° h., at a later stage of practice mentioned that sounds at 90° h. "seemed several times to end up opposite my ear, possibly giving a touch sensation" Yet when the perimeter arm was moved from 135° h. to 90° h., while the variators were sounding, this subject replied that the sound "seemed to move from 30° h. to 45° h.," and that "I felt something blowing on my skin at 45° h. in front of my ear"

In the face of such evidence it seems incredible that tactual sensibility plays any important part in sound localisation. Only a few of my subjects reported its presence, and these agreed that ultimately they discovered the only reliable basis of localisation to consist in differences of timbre and loudness. We seem forced to conclude that the localisation of such tactual sensations is to be regarded as resulting from, instead of giving rise to, determinations of sound localisation *

* It would be rash to assume that auditory stimuli do not give rise to tactual sensations the longest sound waves unquestionably do Nevertheless, it is unlikely that the shortest waves excite tactual sensations, and it seems certain that whatever tactual sensations an auditory stimulus may evoke, they play no part in determining sound localisation.

This view of the illusory function of tactual sensations in sound localisation receives support from other data afforded by my subjects. Many of them, at their early stages of practice, seemed compelled to objectify the sound in tactual visual terms. Thus to one subject a sound at 90° v "appears to come from a central point," while one at 180° "appears to come from different sides as if entering the ear by various rays instead of by a central one." Another subject said "I can't attend to the sensation as such, I have to fancy a motor cycle behind me," or "I fancy myself in a wood with the sound (given at 180°) low down at the end of the path before me" A third subject observed "I give each sound a body, and each is generally circular—a ball" A fourth subject, who had described the variator sounds as generally coming down and hitting her, observed that the buzzer sounds "seemed in many cases to remain in their place and to throw out a sort of pseudopod, like a wriggling worm pulling its tail through" In view of these descriptions, need we hesitate to ascribe localisations of tactual sensation, when they occur, to an inevitable tendency to treat localised sounds as if they were external objects describable in visual and tactual language, and as if they hit the ear, face, vertex, or occiput according to their localisation determined on the basis of timbre and loudness?

IV *Conclusions*

1. The "laterality" of a sound (*i.e.* its estimated position in relation to the median "sagittal" plane) is determined by binaural differences or equality of intensity of the sensation * Experimental changes in the timbre or loudness of a sound make no difference in its laterality

As soon as an infant begins to take notice of sounds, their laterality is at once appreciated There are no trial movements of the head, this way or that, for sounds placed to one side of the median sagittal plane. The reception by one ear of a stimulus stronger than that reaching the other ear at once determines in the infant a movement of the head and eyes to bring the latter towards the source of the sound.

2 On the other hand, even in adult life, the grossest errors are made in determining the incidence of a sound (*i.e.* its estimated position in relation to the horizontal "interaural" line), unless the subject has been practised in the changes in timbre and loudness produced by such changes of incidence, or unless he is allowed to make movements of the head, the effect of which is to vary the timbre and loudness of the sound while it is being heard.

* And, according to Lord Rayleigh, by binaural differences or identity of phase of the sound waves (see, however, footnote to p. 267).

3 The "incidence" of a sound is hence determined by its timbre and loudness. Experimentally produced changes in the timbre or loudness of a sound lead to marked changes in its apparent incidence

4 Tactual sensibility appears to play no part in auditory localisation Localised tactual sensations evoked by auditory stimuli are generally the outcome of interpretations by the subject, resulting from his natural tendency to treat sounds as material objects, and to refer to them a localisation based on solely auditory data

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A Comparative Study of Oxidation by Catalysts of Organic and Inorganic Origin.

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CONTENTS

The present paper is the outcome of the work carried out on the influence of poisoning on apples and potatoes, and its progress has necessitated a

general revision of the oxidase ferments, and in particular a general comparison with metallic oxidases As is well known, oxidases are widely distributed in plants, and are frequently responsible for the changes of colour in extracted plant juices or in plant tissues after death

In the case of the apple and potato, the curious fact that browning took place in pulp killed by immersion in poisonous metallic solutions, but not when killed by heat, demanded special investigation

The Browning of Apples and Potatoes.

It is generally assumed that this is due to the action of an oxidase ferment upon a chromogen present in the pulp cells, such as tannic acid in the apple* and tyrosin in the potato, but the oxidase is not necessarily the same in either case The term "oxidase" in fact rather represents a result than a particular substance, and many "oxidase" actions are not necessarily due to organised ferments or enzymes at all. In a previous paper it has been shown that apple pulp immersed in solutions of metallic poisons may still turn brown, although ordinary ferments are destroyed by such poisons Furthermore, the reasons why the browning takes place on death, but not in the living cell, and not when the cell is killed in certain special ways need investigation Prochromogens or zymogens may exist in the living cell which decompose into interacting chromogen and enzyme on death, or the latter may be kept apart in the living cell by semipermeable membranes which lose their impermeability on death. In the latter case the localisation of the chromogen and enzyme in the cell becomes a problem of special importance

According to Gruss† "Antioxidases"‡ capable of arresting oxidase reactions exist in various plants, and if the oxidase and the "antioxidase" balanced, a chromogen and its oxidase might exist in contact in the living cell and the mode of death might determine whether browning occurred or not Behrens§ considered that the browning of the apple pulp is due to a direct oxidation of tannic acid to form a leathery compound with the proteids of the cell, without the aid of an oxidase

The first point needing full investigation was the influence of poisons on browning, particularly in regard to the time factor and the rapidity of penetration.

* Lindet, 'Compt. Rend,' vol 120, p. 370 (1895)

† 'Biologie und Capillaranalyse der Enzymen,' p 56 (1912)

‡ To avoid possible confusion, the word "inhibitor" may be used instead of this term

§ 'Centralbl. f. Bakt.,' 2 Abth., vol. 4, p. 514 (1898)

The Influence of Poisons on Browning

As is well known, apple and potato pulp if killed by dropping into boiling water, remains colourless in the presence of oxygen for an indefinite length of time, and this according to Bourquelot is due, in the case of the apple, to the destruction of the oxidase responsible for browning When portions of apple pulp are immersed in very dilute sulphuric or tartaric acids, the pulp turns brown, whereas in stronger solutions it remains colourless. This might be due to the stronger acid inhibiting or destroying the oxidase ferment, or preventing its formation if only present as a zymogen in the living cell

Pulp pounded in its own volume of 1-per-cent H_2SO_4 remains colourless and gives no guaiacum test and no distinct decomposition of H_2O_2. If pounded and allowed to brown before adding the sulphuric acid, no oxidase reactions are shown after, but active ones before the addition of the acid Slices of fresh pulp decompose H_2O_2 actively and also turn guaiacum blue If pulp pounded with 10-per-cent H_2SO_4 is neutralised with ammonia and tested, it gives no oxidase reactions. Apparently, therefore, the sulphuric acid acts directly by destroying the oxidase present in the living cells

Pieces of apple pulp immersed in poisonous metallic solutions develop a brown colour on drying, and the same is shown whatever the concentration In 1 and 5-per-cent solutions of lead nitrate, however, the browning is fainter than usual and is mainly confined to the veins. Lead nitrate destroys oxidase ferments and hence apparently the production of browning and the presence of oxidase are not exactly parallel The addition of dilute ammonia rapidly turns the pulp a deeper brown, but the immediate addition of dilute H_2SO_4 or HCl restores the original pale colour Hence the browning produced by ammonia is not quite the same as the permanent brown produced in slowly dying pulp cells If the pulp is soaked in dilute ammonia for some hours, acids will not, however, entirely remove the brown colour

When pieces of pulp are soaked in a poisonous solution, a certain time elapses between the first penetration of poison and the death of each cell, and this time-interval will be greater in the case of deeply seated cells than of superficial ones This is well shown when prepared potatoes are immersed in 5 or 10-per-cent solutions of lead nitrate Chromogen oxidation only takes place towards the inner boundary of the diffusion zone.

To eliminate the time factor the pulp was rapidly pounded in a mortar with the poisonous solution and then tested with guaiacum and H_2O_2. French crab apples were used

Poison	Colour change in air	Guaiacum test	Decomposition of H_2O_2
Untreated	Brown	Blue	Active
1 per cent $HgCl_2$	None	Pale blue	Feeble
1 „ $CuSO_4$	„	Deep blue	Strong
1 „ Pb_2NO_3	„	No blue	None
5 „ Pb_2Ac	Pale greenish or yellowish brown	Pale blue	Fairly active
1 „ $AgNO_3$	Blackens	Strong blue especially with H_2O_2	None
1 „ morphine sulphate	Brown	Pale blue	Feeble
10 „ strychnine	„	„	„
10 „ brucin nitrate	„	„	„
2 „ $BaClO_3$	None	„	„
2 „ $FeCl_3$	Rapidly changing to dark brown	Deep blue	Active
Absolute alcohol	None	Faint blue	Doubtful
Boiled pulp	Nil	Nil	Very feeble

With fresh juice or pulp from potatoes the following results were obtained —

Poison	Colour change in air	Guaiacum test	Decomposition of H_2O_2
5 per cent lead nitrate	Nil	Nil	Nil
5 „ mercuric chloride	„	Blue	Very feeble
5 „ copper sulphate	„	Deep blue	Active
Untreated	Brown	Strong blue	Active
Boiled	Nil	Nil	Very feeble

At first sight these results seem to show that browning is not closely related with the presence of the oxidase With absolute alcohol, however, if the pulp is allowed to stand for a short time the oxidase reactions entirely disappear Apparently the alcohol first weakens and then destroys the oxidase and the oxidation of tannic acid seems to require a more powerful oxidase action than is necessary to produce a blue with guaiacum

Inorganic Oxidases.

In addition the influence of the metallic poisons must be taken into account. According to L. Meyer,* salts of manganese such as the chloride and sulphate can act as strong oxidases, and salts of copper, iron, and cobalt have the same power but progressively decreasing, whilst the least oxidase action is shown by salts of nickel, zinc, cadmium, and magnesium It is not, however, clear as to whether a strong metallic oxidase oxidises all substances

* 'Ber Chem Gesell,' vol. 20, p. 3085 (1887)

capable of oxidation with the same relatively greater intensity than does a feeble oxidase, or whether a strong oxidase to one substance may be a feeble oxidase to another as in the case of the organic oxidases. Other points needing elucidation are as to whether oxidase action is solely due to the metallic base, and what influence a metal such as iron will have when present as an acid. Also as to whether peroxide of hydrogen only accelerates oxidase action when the oxidase salt decomposes it. In the following table the results of a series of tests are given using guaiacum, ursol tartrate,* hydroquinone, pyrogallic acid, gallic acid, tannic acid, and tyrosin as oxidant substances. In order to render possible a comparison of the relative activities in each case, the oxidase was present in one-tenth the molecular concentration of the oxidant, except in the case of the guaiacum, where the alcoholic solution is best allowed to float as a thin layer on the oxidase solution.

The exposure to air was continued for one day, and if the colour was still the same as the test solution the result is given as nil. A rapid reaction is indicated by three positive signs (+ + +), slower ones taking one or more hours to become distinctly perceptible by two signs (+ +), a very slow one taking the full 24 hours by one (+). In the case of guaiacum, owing to the mode of application of the test, the time factor does not enter to the same extent, but a difference in the strength of the oxidase is indicated by the depth of the blue coloration (strong = + + +, weaker blue = + +, feeble blue = +).

	Katalase action	Guaiacum	Ursol tartrate.	Pyrogallol	Hydro-quinone	Gallic acid	Gallotannic acid.	Tyrosin.
Cupric chloride		+ +		+		+		
Do + H_2O_2	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+	
Cupric sulphate							+	
Do + H_2O_2		+ +	+	+ + +	+ + +	+ + +	+	
Copper oxychloride				+				
Do + H_2O_2		+	+	+ +	+ +	+ + +	+	+
Copper acetate and subacetate		+		+	+			
Do + H_2O_2	+*	+ + +	+	+ +	+ +	+ +	+	+
Ferric chloride		+ + +	+ + +	+ +				
Do + H_2O_2	+ + +	+ + +	+ + +	+ + +	+ + +†	+ + ?	+ ?	+
Ferrous sulphate								
Do + H_2O_2	+ + +‡	+ +	+ +	+ +	+ +	+ + ?	+ ?	+

* $Cu_2O_2Cl_2 4H_2O$

† Flat black shining needles separate out on standing in the cold, but no distinct oxidase action is shown

‡ Liquid yellow, then slowly brown precipitate

* Paraphenylenediamine tartrate

	Katalase action	Guaiacum.	Ursol tartrate	Pyrogallol	Hydroquinone.	Gallic acid	Gallotannic acid	Tyrosin
Ferrous chloride		+						
Do $+H_2O_2$	+ + +*	+ +	+ +	+ +	+ +	+ +	+	+
Potassium ferrocyanide								
Do $+H_2O_2$	+†	+ + +	+ +	+ +	+	+ +		
Potassium ferricyanide		+	+ +	+ +		+		
Do $+H_2O_2$		+ + +	+ + +	+ + +	+ +	+ +		
Manganese chloride ($MnCl_2$)				+				
Do $+H_2O_2$		+?	+	+ +	+	+		
Manganese sulphate ($MnSO_4$)								
Do $+H_2O_2$		+ +	+ +	+	+	+		
Potassium permanganate		+ + +	+ + +	+	+	+ +		+ +
Do $+H_2O_2$	+ + +	+ + +	+ + +	+ +	+ +	+	+	
Black oxide of manganese		+ +	+ + +	+ + +	+	+ +		
Do $+H_2O_2$	+ + +	+ + +	+ + +	+ +	+ +	+		
Chromium chloride								
Do $+H_2O_2$	Nil	Nil	+ +	+ +	+ +	+	+	+
Chromic acid		+ + +	+ +	+ + +	+ +	+ + +	+ + +	
Do $+H_2O_2$‡	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+
Potassium bichromate		+ + +	+ +	+ +	+	+ + +		
Do $+H_2O_2$‡	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +	+
"Neutral" potassium phosphate§				+				
Do $+H_2O_2$	Nil	Nil	+	+	+			
Nitric acid		+ + +	+	+ + +	+ +	+ +	+	
Do $+H_2O_2$				+ +		+		
Lead acetate								
Do $+H_2O_2$	+	+ + +	+	+ +	+	+	+	

* Ferric salt formed, hence the oxidase reactions with H_2O_2 the same as with ferric chloride

† H_2O_2 converts ferrocyanide partly into ferricyanide Hence ferrocyanide and H_2O_2 give similar oxidase reactions to the ferricyanide Similarly traces of ferricyanide appear slowly in a solution of ferrocyanide exposed to light and the liquid becomes a deeper yellow, while ultimately prussian blue separates out According to Sarthou ('Journ Pharm Chim,' vol 1, p 482, 1900), the bark of *Schinus molle* contains a ferment "schinoxidase" which converts potassium ferrocyanide into ferricyanide It is, however, very doubtful in this case that we are dealing with an oxidase ferment at all

‡ With dilute solutions the colour change with hydroxyl is easily distinguished from the oxidase change by using controls A mixture of dilute CrO_3 and H_2O_2 becomes colourless again on long standing

§ Made by adding potassium carbonate to a boiling solution of acid potassium phosphate until imperceptibly acid or alkaline to litmus

The foregoing Table shows clearly that an inorganic oxidase is not necessarily a "katalase," nor a katalase an oxidase, and that hydrogen peroxide may accelerate the oxidase action of substances incapable of decomposing it

In the case of nitric acid, chromic acid and potassium permanganate the

oxidation is, in part at least, a direct one and hydrogen peroxide diminishes the oxidising action. If hydrogen peroxide is added to very dilute potassium permanganate, a colourless liquid is formed and the evolution of oxygen soon ceases With stronger solutions the liquid is brown, and contains an oxide capable of continuous oxidase and katalase action

The Tables show further that chromium and iron can act as oxidases when present in the form of acids

In general a feeble oxidase acts feebly on all the substances tested, and the order of sensitivity to oxidases is guaiacum, ursol tartrate, pyrogallol, hydroquinone, gallic acid, gallotannic acid, tyrosin There are, however, various exceptions. Thus chromium chloride and manganese chloride give a blue with guaiacum, and copper sulphate does the same in the presence of H_2O_2, but all three give direct oxidase reactions with ursol tartrate, pyrogallol, etc Where there is a strong tendency to precipitation between the oxidase and oxidant as in the case of lead acetate or of ferric chloride and hydroquinone, the oxidase action may be retarded or prevented. In the case of potassium phosphate the feeble oxidase properties are evidently due to the phosphoric acid and not to the potassium. Further, the chlorides, nitrates or sulphates of the same metal are not necessarily equally powerful oxidases, chlorides apparently surpassing sulphates (see copper) and nitrates chlorides. Thus cobalt chloride shows no oxidase properties with or without H_2O_2. Cobalt nitrate slowly browns pyrogallol and hydroquinone in the presence of H_2O_2 but is inactive to guaiacum, ursol tartrate, and tannic acid.

Lead nitrate shows no oxidase action, whereas lead acetate exhibits a peroxidase action. Yellow potassium chromate has similar oxidase properties to potassium bichromate except that it causes tannic acid to brown rapidly in the absence of H_2O_2, probably owing to the alkaline nature of the basic chromate.

The Nature of Oxydases.

The fact that certain plant oxidases contain oxidase metals, such as manganese in laccase, has long been known and certain oxidases, as for instance, tobacco oxidase, can be boiled without being destroyed. Woods* considers that this is due to the oxidase existing as a zymogen from which on cooling the oxidase is reproduced. The supply of zymogen can, however, hardly be unlimited, and, since the boiling can be repeated more than once without destroying the oxidase, it must itself be resistant to heat

According to Bach and Chodat† the oxidases form three distinct groups of ferments, namely —

* 'Bull. U S. Dept. Agric.,' vol. 18, p. 17

† 'Biochem. Centralbl.,' 1903, p. 141.

(1) Oxygenases, proteins which absorb molecular oxygen forming peroxides;

(2) Peroxidases, which increase the oxidising power of peroxides and can only act in their presence;

(3) Katalases, which destroy peroxides with an evolution of oxygen.

An oxidase which turns guaiacum blue without hydrogen peroxide being added is a mixture of ferments of the first and second class. Thus Bach* considers tyrosinase to be a mixture of a specific oxidase and a specific peroxidase.

Moore and Whitley† conclude that all oxidases are peroxidases acting in the presence of a peroxide, such as may be present in certain solutions of guaiacum, or an organic peroxide derived from the juice of the plant tested. The peroxides can be removed from the juices and from guaiacum by adding animal charcoal and filtering, and they are destroyed by heating to 55°–60° C. for several hours. After such treatment potato juice will only give a blue with guaiacum on adding H_2O_2.

As a matter of fact the action is merely to attenuate the oxidase, so that the addition of an accelerator such as H_2O_2 is necessary to render the oxidation of the guaiacum perceptible. The facts that potato juice decomposes peroxides strongly, and that the juice and pulp give negative results when tested with decolorised magenta, and show no effervescence until H_2O_2 is added, are hardly in accord with Moore and Whitley's explanation, and a study of the preceding Tables shows that, so far as metallic oxidases are concerned, too much importance can easily be attached to the influence of peroxide of hydrogen on oxidase action. Thus in some cases the same substance may be a katalase, an oxidase, and a peroxidase. In other cases the same metallic salt may be an oxidase to one reagent, a peroxidase to another, ineffective to another, and may or may not at the same time be a katalase. Finally, the mere addition of H_2O_2 may convert a weak oxidase ("peroxidase") into a stronger one, which will then act in the absence of H_2O_2 (ferrous salts and potassium ferrocyanide).

Moore and Whitley found that hydrochloric acid of 1/800 normal concentration nearly destroyed potato oxidase, and quite destroyed carrot oxidase, while sodium hydrate of similar concentration had no effect. On the other hand, Na_2HPO_4 had a stronger destructive action than NaH_2PO_4. The reaction may, however, be prevented without the oxidase being destroyed. Thus the addition of hydrochloric acid (or sulphuric) to ferric chloride removes its power of giving a blue with guaiacum, while tartaric and citric

* 'Berichte,' vol. 39, No. 10, p. 2126 (1906).

† 'Biochem. Journ.,' vol. 4, p. 136 (1909).

acids hinder or decrease the blue reaction, which is however given strongly even in the presence of 1-per-cent oxalic acid Hydrogen peroxide is partially antagonistic to this action, and it is possible to obtain acidified solutions of ferric chloride which will give a blue when peroxide of hydrogen has been added, but not when it is absent The effect probably depends upon the ionic condition of the iron in solution, but the disappearance of an oxidase reaction on the addition of acid does not necessarily mean that the oxidase has been destroyed, any more than ferric chloride is destroyed by the addition of hydrochloric acid Further, in Moore and Whitley's experiment with expressed potato sap heated to 55° C for some hours, the sap becomes more acid, and this might in itself be a sufficient explanation of why an addition of peroxide of hydrogen then becomes necessary to obtain an oxidase reaction

Further, the case of cobalt chloride and ammonia shows that the addition of alkali to certain plant oxidases might greatly increase their oxidase activity or might convert a non-oxidase combination into an oxidase one Thus, with a small quantity of ammonia, cobalt chloride forms a green precipitate, slowly oxidising to brown; but with slight access of ammonia a nearly colourless liquid is formed, oxidising to brown from the surface If the two liquids are diluted, the latter gives a blue with guaiacum directly, and the former an intense blue on adding H_2O_2, but without H_2O_2 no blue is given, or only a faint trace on long standing In other words, an oxidisable substance can act as an oxidase, and ammonia, by accelerating the rate of auto-oxidation, also increases the intensity of oxidase action and converts a peroxidase into an oxidase

According to Porodko,* *per* salts (-ic) of iron, copper, manganese, and chromium give a blue with guaiacum in the absence of peroxide of hydrogen, *proto* salts (-ous) only when it is present This is, however, by no means a general rule Ferrous chloride gives a blue without H_2O_2, but not cupric sulphate or manganic chloride in moderate dilution Lead nitrate gives no blue in the presence or absence of hydrogen peroxide, while lead acetate gives a strong blue in its presence Further, the addition of H_2O_2 converts the non-oxidase ferrous sulphate or potassium ferrocyanide into ferric compounds, which give a blue with guaiacum in the absence of hydrogen peroxide

Oxidase Sensitisers and Inhibitors.

Various neutral salts may exert a powerful action on metallic oxidases either as sensitisers or retardants Thus an old test for a soluble copper salt given by Purgotti is · Add salt and pour on top an alcoholic solution of

* 'Bot Centralbl,' Beihefte 2, vol. 16, p 1 (1904)

guaiacum—strong blue colour Either sodium or potassium chloride will cause copper sulphate to give as deep a blue as in the presence of H_2O_2, *i.e.* converts it from a "peroxidase" to an oxidase. The action is not solely due to cupric chloride being present in the mixed solution, since the blue is deeper than with cupric chloride alone The intensity of the action decreases slowly with increasing dilution It is given by a dilution of 1 grm. of copper sulphate in 100,000 c.c. of water, faintly in a dilution of 1 in 250,000, very faintly by 1 in 100,000, and not at all in a solution of 1 in 10,000,000, *i.e.* guaiacum is about as sensitive in the presence of salt to the oxidase action of copper sulphate as the pulp cells of apples are to its poisonous action

Sodium and potassium phosphates are also able to act as sensitisers to such oxidases as potassium ferricyanide, and the influence of a sensitiser may show with some but not necessarily with all test substances (oxidants) The accelerating action of phosphates is particularly marked with tannic acid, if sufficient is added to leave a clear solution An excess produces a purplish white precipitate and naturally interferes with oxidation Acid potassium phosphate is a less active oxidase sensitiser than the neutralised solution of the same salt Neutral potassium phosphate accelerates the oxidation of tannic acid by potassium permanganate, but not by black oxide of manganese, and it acts as a retardant to those soluble metallic oxidases which it precipitates

Water itself may act as a sensitiser as well as an oxygen carrier Thus, if potassium ferricyanide is dissolved in pure boiled glycerine and guaiacum dissolved in absolute alcohol added, even after long standing only a faint blue or none at all appears at the junction of the two liquids, which rapidly intensifies on adding a little water Nasse and Fram* have even gone so far as to ascribe the oxidation entirely to the hydroxidation of water without the presence of free oxygen being necessary, but Porodko† has shown that this is not the case

The addition of a neutral solution of potassium phosphate to potassium ferrocyanide does not cause any ferricyanide to appear, but causes it to behave as a weak oxidase to guaiacum, ursol tartrate, pyrogallol and hydroquinone, and as a "peroxidase" to gallic acid, tannic acid and tyrosin The action in the presence of hydrogen peroxide is, however, in part due to its partial conversion into ferricyanide Neutral potassium phosphate intensifies the oxidase reaction of potassium ferricyanide and converts it from a non-oxidase to tannic acid and tyrosin into an oxidase to the former and a "peroxidase" to the latter

* 'Pflüger's Archiv,' vol. 63, p 203 (1896)

† 'Bot. Centralbl,' Beihefte, vol. 10, p. 1 (1904)

	Katalase action	Guaiacum	Ursol tartrate	Pyrogallol	Hydro-quinone.	Gallic acid.	Gallotannic acid.	Tyrosin.
Acid potassium phosphate								
Do + H_2O_2	+		+					
Neutral potassium phosphate				?				
Do + H_2O_2			+	+	+			
Potassium ferrocyanide and acid phosphate						?	?	
Do + H_2O_2	+	+ + +	+ +	+		+	+	*
Potassium ferrocyanide and neutral phosphate		+	+ +	+				
Do + H_2O_2	+	+ +	+ + +	+ +	+ +	+ +	+ +	+
Potassium ferricyanide and acid phosphate		+ +	+	+	?	+ +	+ +	
Do + H_2O_2		+ + +	+ + +	+	+	+	+ +	
Potassium ferricyanide and neutral phosphate		+ + +	+ + +	+ +	+	+ + +	+ + +	
Do + H_2O_2	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+

* Yellowish colour due to formation of ferricyanide

To some extent acid potassium phosphate and peroxide of hydrogen are antagonistic in their action on potassium ferricyanide, and hence the oxidation produced when both are added may be no greater or even less than when either is added singly

In the case of tannic acid, the addition of sodium or potassium phosphate seems not so much to accelerate the action of the oxidase as to render the tannic acid more liable to oxidation The chlorides and bromides of sodium and potassium act as strong sensitisers to certain metallic oxidases but not to others, and even with the former the sensitising action is not the same to all oxidants By themselves these salts exhibit no oxidase properties with any of the oxidants mentioned

A detailed comparison is given beneath of the influence of potassium chloride upon the oxidase action of relatively inert oxidase salts such as

	Katalase action.	Guaiacum	Ursol tartrate	Pyrogallol	Hydro-quinone	Gallic acid	Tannic acid	Tyrosin
Copper sulphate and sodium chloride	+ +	+ + +		+		+	+	
Ferrous sulphate and potassium chloride	+ +	+ *		+		†		
Chromium chloride and sodium chloride		+		+				

* Fading again on long standing

† A pale violet colour darkening from the surface possibly owing to oxidation to ferric salts

copper sulphate, ferrous sulphate and chromium chloride, which act as oxidases in the presence of hydrogen peroxide but not in its absence.

Copper sulphate, in the presence of salt and H_2O_2, rapidly causes an oxidase browning in tannic acid, a slight browning is slowly produced with tyrosin, and a full colour sequence with ursol tartrate The oxidase reactions of copper sulphate and H_2O_2 with pyrogallol, hydroquinone, and gallic acid are approximately the same in the presence as in the absence of salt

In the presence of H_2O_2 salt slightly accelerates the oxidase action of chromium chloride on hydroquinone and pyrogallol, and a deep blue is given with guaiacum, particularly if bromide is used instead of chloride, but a weaker blue if no chloride or bromide is present In general sodium and potassium bromides are slightly stronger sensitisers than the chlorides, the iodides are less active* and the fluorides still more so or may even exercise the reverse action

Copper acetate, ferrous chloride and potassium ferricyanide, which slowly give a pale blue with guaiacum in the absence of H_2O_2, give a stronger blue rapidly in the presence of salt nearly as well as when H_2O_2 is added Salt is, however, unable to produce a blue in the absence of H_2O_2 with manganese sulphate or chloride, with copper oxychloride or with potassium ferrocyanide.

The relative mass of the oxidase and sensitiser is of importance Thus if equal masses of ferrous sulphate and of KCl, KI, or KF are present, and the solutions fairly strong, no blue is given with guaiacum, but if the ferrous sulphate is present in relatively dilute solution, a rather pale blue is given with KCl, weaker with KBr and KI, and faint or imperceptible with KF. Hence if the oxidase and sensitiser are not present in the proper proportions some oxidase actions may be prevented or overlooked Copper sulphate, however, even when present in excess gives direct oxidase reactions in the presence of sensitisers, possibly because unlike ferrous sulphate it has no tendency to auto-oxidation On the other hand, a sensitiser does not act with all oxidase tests Thus neither KI, KBr, KCl nor KF, whether relatively dilute or concentrated, give ferrous sulphate any oxidase action on ursol tartrate or hydroquinone

The double fluoride of sodium (NaFHF) inhibits the oxidase action of ferric chloride on guaiacum, ursol tartrate, pyrogallol, hydroquinone and tyrosin, and also its power of decomposing H_2O_2. It strongly retards the oxidase action of potassium ferricyanide and of manganese sulphate and H_2O_2, and although a blue is still given with guaiacum it is much paler. A rather weaker retarding action is also exercised upon the oxidising action of potassium permanganate

* They cannot be tried with copper, owing to the precipitation of the latter, or used in the presence of H_2O_2, owing to the decomposition of the iodide by the peroxide.

and upon its power of decomposing H_2O_2, the solution in the latter case remaining clear instead of turning brown.

The single fluoride (NaF) is also capable of acting as an antagoniser, particularly shown in the case of ferric chloride with guaiacum and hydroquinone, while with pyrogallol a violet-blue is given instead of dark brown On the other hand the addition of sodium fluoride to potassium ferricyanide increases all its oxidase reactions except to pyrogallol, where a retardation is shown, and renders it a weak oxidase to tannic acid and a "peroxidase" to hydroquinone

If a dilute solution of lead nitrate is added to a dilute solution of copper acetate and salt in such proportion that two atoms of chlorine are present to each atom of lead, a pale blue is still given with guaiacum, but none if the lead nitrate is in excess, although in neither case is any precipitate formed Lead nitrate might, therefore, be regarded as an antagoniser to copper acetate and sodium chloride as a sensitiser An excess of lead nitrate prevents copper acetate giving a distinct blue by itself, but a blue is still given in the presence of H_2O_2

In the case of potassium permanganate the addition of sodium fluoride does not affect the guaiacum test or the decomposition of H_2O_2, slightly accelerates the oxidation of tannic acid (in the presence of H_2O_2) and of ursol tartrate, and distinctly retards the oxidation of pyrogallol and hydroquinone The same substance may, therefore, be a sensitiser to one oxidase and an antagoniser to another, while the action may vary according to the substance oxidised.

Sensitisers and Antagonisers to Plant Oxidases

It is well known that diastase acts as an oxidase to guaiacum and the same applies to other ferments Plant oxidases, however, appear to be more specific and less generalised in their action than are metallic oxidases. Hence it is of importance to determine to what extent the specific peculiarities of certain plant oxidases can be ascribed to the presence of accompanying sensitisers and antagonisers or inhibitors.

In the following Table a general comparison is given between a few of the common plant ferments and oxidases. In the first four cases watery solutions were used, in the last two cases thin slices of potato and apple were rapidly dried *in vacuo* after squeezing out the sap and were pounded to powder. The powder was added to the test solution.

The chief peculiarities are that potato oxidase acts strongly on tyrosin and feebly or not at all on tannic acid or on ursol tartrate in the absence of hydrogen peroxide, while apple oxidase, which is generally weaker, acts strongly on tannic acid and ursol tartrate but not at all on tyrosin.

	Katalase action	Guaiacum.	Ursol tartrate	Pyrogallol	Gallic acid	Tannic acid.	Tyrosin.
Malt diastase		+					
Do $+H_2O_2$	+	+ + +	+	+			
Papain				+			
Do $+H_2O_2$		+	+	+			
Popsina porci							
Do $+H_2O_2$		+					
Pancreatin							
Do $+H_2O_2$							
Potato powder		+ + +		+			
Do $+H_2O_2$	+ + +	+ + +	+ +	+ + +	+ +		
Apple powder		+ + +	+ +	+		+	
Do $+H_2O_2$	+ +	+ + +	+ + +	+ +	+ +	+ +	

Neither the chlorides nor the phosphates of potassium or sodium accelerate the oxidase action of malt diastase. If anything the addition of malt diastase to potassium phosphate appears to slightly retard the feeble oxidase action of the latter, especially to ursol tartrate Sodium or potassium bromide enfeebles the blue reaction of diastase with guaiacum and H_2O_2

The watery or glycerine extract of apples, or the pounded pulp, gives a reaction with ursol tartrate in the absence of hydrogen peroxide, but not potato pulp or its oxidase extract Living slices of potato give a surface reaction after a day's immersal and the pounded pulp or oxidase extract gives a faint reaction on long standing According to Moore and Whitley this would be due to its being a "peroxidase" only able to act when peroxidases developed on the surface If so it is difficult to understand why both apple and potato oxidase and pulp should at once give a blue guaiacum free from peroxide Rapidly expressed and boiled apple sap contains no trace of hydrogen peroxide and has no perceptible action on ursol tartrate, but if it is added to pounded potato pulp or oxidase extract, the latter now gives a distinct and fairly rapid oxidase action with ursol tartrate, which is not accelerated further by the addition of salt or other sensitisers. Evidently apple sap contains a sensitiser which is absent or deficient in potato pulp and which is not an acid, for no reaction with ursol tartrate is produced by the addition of dilute hydrochloric, oxalic, citric, malic, or tartaric acids to the potato pulp.

Apple ash is rich in potassium, which occurs mainly as phosphate and carbonate. Acid potassium phosphate and normal potassium carbonate have little or no accelerating action on potato oxidase. If they are mixed so as to produce a neutral solution and a small amount added to potato oxidase, the latter will oxidise both ursol tartrate and tannic acid,

on which previously it had little or no action. The readiness with which apple pulp or its extracted oxidase oxidises ursol tartrate and tannic acid is therefore apparently due to the presence of a phosphatic sensitiser. Potato pulp contains phosphates in less amount and in less readily soluble form but if a living slice of potato is immersed in boiled apple sap, a brown layer of oxidised tannic acid slowly forms on the surface where the oxygen and tannic acid come into contact with oxidase and traces of phosphates under optimal conditions for progressive oxidase action In the living cell the permeability of the cell membrane may determine whether an oxidase and its sensitiser come into contact simultaneously, singly or not at all with an oxidant substance

The Relation between the Action of Metallic Oxidases and that of an Enzyme

This question has been fully investigated by H E and F Armstrong, in a long series of papers by themselves and pupils, so far as acids and hydrolysing enzymes are concerned A few data in regard to certain inorganic oxidases are given beneath, and firstly in regard to the dilution at which a perceptible action is shown Ferric chloride will give a perceptible blue with guaiacum down to a concentration of 0 0001 per cent., while copper sulphate in the presence of salt gives a faint blue down to 0 00001 per cent With a 1-per-cent solution of ursol tartrate in the presence of an equal volume of 1-per-cent. hydrogen peroxide a distinct acceleration of oxidation is shown down to a concentration of 0 0001 per cent. of ferric chloride (0·00003 per cent in total solution) The oxidation of hydroquinone in the presence of hydrogen peroxide is accelerated by the addition of an equal volume of 0 001-per-cent ferric chloride but not perceptibly so by lesser dilutions.

One property of an oxidase enzyme is that it may transfer oxygen from one labile compound to another. In the following experiment equal volumes of 1-per-cent hydroquinone and of ferricyanide of potassium and hydrogen peroxide were mixed, and the time taken to reach a standard shade of brown noted The top line of the Table gives the concentration of the substance whose amount was varied The lower rows give the time in minutes required to carry the oxidation to the same stage in each case, and the figures in brackets are the products of the time of reaction multiplied by the concentration of the variant substance. In experiment A, to each 5 c c of 1-per-cent. hydroquinone, and of 1-per-cent. hydrogen peroxide, 5 c.c. of potassium ferricyanide of varying concentration were added In experiment B the hydrogen peroxide was in diminishing concentration, and in C, both the hydrogen peroxide and potassium ferricyanide decreased in concentration correspondingly.

Oxidation of Hydroquinone by Potassium Ferricyanide and Hydrogen Peroxide

	Concentration of variant substance (per cent.)								
	1	0·5	0·25	0·1	0·05	0·01	0·005	0·001	0·0001
Experiment A	17 (17)	20 (10)	23 (5·6)	32 (3·2)	38 (0·76)	48 (0·48)	56 (0·24)	2378 (2·38)	Trace only
Experiment B	17 (17)	22 (11)	38 (9·5)	66 (6·6)	Incomplete after 3 days	Faint after 1 week	Trace only		
Experiment C	17 (17)	21 (10·5)	32 (8)	1740 (174)	Incomplete after 1 week	Faint after 1 week	Trace only		

The experiments show that in the presence of abundance of hydrogen peroxide an oxidase action is perceptible down to a concentration of 0·001 per cent. of potassium ferricyanide (1 grm. in 100,000 c.c. of water), and that the relative oxidase activity increases with dilution down to a concentration of 0·005 per cent., beyond which it rapidly decreases to nil, the limit being possibly set by extreme conditions of mass action.

Apple Oxidase.

In regard to the influence of KI, KCl, KBr, KF, in all cases the fluoride acted more or less strongly as an antioxidase, while the other salts for the most part exercised a slight retarding influence in the order given, although this was imperceptible with the guaiacum test. The chloride feebly and the bromide more strongly accelerated the decomposition of H_2O_2, and also the oxidation of hydroquinone in the presence of H_2O_2.

When in excess, all four salts strongly retard or even prevent the browning of pounded apple pulp, but without destroying the oxidase. On washing away the excess of the salts and adding H_2O_2, a blue is given with guaiacum and the KBr pulp turns rapidly, the KCl pulp slowly, and the KF pulp very slowly brown. Pulp pounded with 2-per-cent. barium chloride remains colourless, and after three hours, on adding dilute H_2O_2, a feeble evolution of gas is shown and the pulp browns rapidly, but not if previously boiled. If the pulp is washed with water, filtered, and the residue pounded up with a little fresh water, it is able to actively decompose H_2O_2, gives a distinct blue with guaiacum, and on exposure to air slowly browns. Barium chloride, therefore, does not destroy the oxidase but acts as an antagoniser, and

peroxide of hydrogen is able to partially suspend its inhibitory action. In the presence of barium chloride, apple oxidase acts as a "peroxidase," in its absence, as an oxidase

Strong peroxide of hydrogen destroys the oxidase, and hence pieces of fresh pulp when immersed in a strong solution of pure peroxide remain colourless or show a faint browning along some of the veins The peroxide, as it penetrates, destroys the oxidase in the protoplasm before it comes into contact with tannic acid If the pulp is pounded with strong peroxide, the browning is somewhat retarded, but still takes place, since the oxidase, tannic acid, and peroxide are in contact simultaneously and react before the oxidase is destroyed Dilute peroxide accelerates the browning of pounded pulp

Lead acetate appears at first sight to be incapable of preventing the browning of pounded apple pulp The pulp darkens to yellowish or greenish brown, and even after 24 hours retains a distinct but enfeebled power of decomposing hydrogen peroxide and turning guaiacum blue The darkening is, however, partly due to the precipitation of yellowish-white lead tannate and the retention of the power of turning blue is easily explained, for lead acetate itself gives a strong blue with guaiacum in the presence of hydrogen peroxide, and can therefore act as an oxidase Any poison which destroys the oxidase also removes the power of turning brown, and the pulp of apples turns brown when soaked in bulk in metallic poisons, because the slowness of penetration allows the cell to be killed and browning to occur before the oxidase is destroyed Barium chloride, however, inhibits oxidation without destroying the oxidase ferment.

The Chromogen of the Apple

In the apple the chromogen is known to be a form of tannic acid. In a previous paper it was shown that tannic acid vacuoles appeared in the protoplasm of pulp cells immersed in methyl blue or ferric chloride, so that the assumption that the whole of the tannic acid was present in the cell sap did not appear to be correct No such vacuoles could, however, be detected in living pulp cells, or in the protoplasm of cells killed by heat prior to staining Further, although pulp from which most of the sap has been expressed turns, if anything, darker with $FeCl_3$ than before, this may be merely because the tissue is more compacted In addition, if slices of pulp are subjected to very strong pressure between wads of filter paper until the pulp is quite dry, on moistening with water the pulp remains colourless or the veins may turn brown, and no distinct tannic acid reactions are given with $FeCl_3$, KCN, or iodine and ammonia. Microscopic examination shows

that the protoplasm is still present in the pulp cells, and very careful testing shows that a trace of tannic acid may adhere to it. The colourless pulp from which the sap has been removed, after pounding with water, gives distinct oxidase reactions. Even after repeated extractions of pounded pulp with absolute alcohol, a little tannic acid adheres to the pulp, which turns green and then brown with ferric chloride, but remains colourless in air owing to the destruction of the oxidase by the alcohol. Apparently, therefore, the tannin vacuoles which may appear in the protoplasm are factative and are due to the methyl blue or ferric chloride meeting tannic acid in the dying protoplasm. They are possibly analogous in origin to the vacuolation which may be produced in dying protoplasm by various chemical agencies. Using concentrated solutions of methyl blue or ferric chloride they do not appear in the surface cells but only in those at a certain depth. Evidently it is a condition for their formation that the cell should die slowly.

In regard to the form of tannic acid present, this is not gallotannic acid, but is more closely allied to mangrove tannin. Thus with calcium hydrate it gives white turning red and not blue. As it gives green with ferric chloride instead of blue or black, it is presumably a catechol yielding tannin and not a pyrogallol tannin. The green given by the expressed sap or pounded pulp with ferric chloride rapidly changes to brown, especially if the material is nearly neutralised. Pounded pulp, allowed to brown, darkens to almost black with $FeCl_3$, and shows no green colour at first, but this does not indicate a production of gallotannic acid by oxidation, but may be due to the superposition of the two colours.

If a dilute solution of gallotannic acid (0.05–0.1 per cent.) is divided into three parts, A, B, C, and C is saturated with common salt, on adding an excess of 10-per-cent. ferric chloride to B and C a green liquid is formed, whereas with a drop only of ferric chloride A gives a blue-black liquid. On boiling, A forms a blue-black precipitate, B forms a brown liquid, and C forms a green or yellowish-green liquid. The blue-black colour reaction of gallotannic acid with ferric chloride is therefore capable of various modifications.

Gallotannic acid gives a blue-black precipitate with ferric chloride in the presence of oxalic acid. If ferric chloride is added to a slight excess of gallotannic acid and apple sap added to the blue-black liquid, it turns green, just as the sap does with $FeCl_3$, but on standing the first liquid takes a bluish tinge, while the second becomes brown. Hence, apparently, the different reactions are partly due to differences between gallotannic acid and the tannic acid of apple pulp and partly to the substances which accompany the latter, but are not entirely due to the presence of free organic acid in the

sap There is nevertheless a close correspondence between the behaviour of gallotannic acid and of the apple greening tannin to metallic oxidases.

Thus dilute gallotannic acid solutions give a turbid liquid with copper sulphate, but in the presence of salt a clear liquid After one day a pale brown precipitate separates out from both liquids With peroxide of hydrogen gallotannic acid is unaffected, but in the presence of copper sulphate, sodium chloride, and hydroxyl the gallotannic acid immediately develops a strong brown colour

Clear boiled apple sap shows practically identical reactions. Colourless pulp pounded with copper sulphate remains unchanged, with copper sulphate and salt it develops a faint, barely perceptible, brownish tinge in the parts exposed to air, and with copper sulphate and hydroxyl it develops a pale brownish tinge, which is immediately intensified on adding salt Pounded up with copper sulphate, salt, and hydroxyl, boiled apple pulp is browned as deeply or almost as deeply as by the full action of the natural oxidase, whereas potato pulp is unaffected

The Chromogen of the Potato

The chromogen of the potato appears to be not a tannin compound, but some substance related to tyrosin Freshly pulped potatoes acquire a purplish-brown tinge in air, removed by washing and squeezing and returning to a less and less extent with each washing on standing. The chromogen appears to be dissolved in the cell sap and the product of oxidation to be soluble in water In the apple when the tannin is oxidised inside the cell, it is rapidly absorbed and permanently retained by the protoplasm.

No distinct traces of tannic acid can be detected in resting potatoes by ferric chloride or other tests In 10-per-cent. sodium hydrate, potato pulp becomes transparent but remains practically colourless

If tyrosine is added to fresh potato pulp, or to a diluted glycerine extract, a purplish-brown colour is given more rapidly and deeply. This colour is also soluble in water, and hence presumably the chromogen is tyrosin

Boiled potato pulp or sap remains colourless. Cubes of pulp treated with absolute alcohol remain colourless both in air and in water. The alcoholic extract is pale yellow, and has no oxidase properties If evaporated *in vacuo* and water and a glycerine extract of oxidase added no change of colour occurs. Treatment with absolute alcohol seems, therefore, to either destroy or precipitate both oxidase and chromogen. If the pulp from alcoholic extraction is pounded with water and a glycerine extract of oxidase added the pulp remains almost colourless but the supernatant liquid turns

purplish-brown Hence the chromogen after precipitation by absolute alcohol can be dissolved again in water The expressed sap from potatoes turns black in a day If boiled, treated with HCl or absolute alcohol a dirty white or grey precipitate is thrown down, and in the case of the absolute alcohol the supernatant liquid is colourless and devoid of both chromogen and oxidase

Potato Oxidase

Potassium chloride and bromide feebly and potassium fluoride strongly retard its oxidase action on ursol tartrate and H_2O_2, on hydroquinone and H_2O_2, and on pyrogallol and tyrosin. The addition of 5-per-cent lead acetate to the glycerine extract throws down a bulky white precipitate which gives no blue with guaiacum, but shows an active power of decomposing H_2O_2, and in the presence of the latter gives a distinct blue with guaiacum Lead acetate itself, however, gives a peroxide reaction with guaiacum Potato oxidase by itself is unable to oxidise tannic acid even in the presence of H_2O_2, but if a little neutral solution of sodium phosphate is added, the liquid turns slowly pale brown in the absence of H_2O_2, and more rapidly a darker brown in its presence

Potato oxidase is peculiar in the readiness with which it oxidises tyrosin, which most metallic oxidases only oxidise slowly and usually only in the presence of H_2O_2 Possibly this peculiarity is due to the presence of a specific sensitiser in the potato Potassium phosphate appears to act as a feeble sensitiser to the oxidase action of potassium ferricyanide on tyrosin, but the nature of the sensitiser in the potato is doubtful That such may be present is indicated by the fact that potato extracts may sometimes be obtained apparently capriciously, which while still reacting strongly to guaiacum only react feebly to tyrosin and conversely. This is particularly the case when partially sprouted tubers are used and fractional extractions made

The Extraction of the Oxidase.

The oxidase of the potato is either more active or more abundant than that of the apple, whereas the chromogen of the apple is more abundant than that of the potato Absolute alcohol not only does not extract the oxidase but destroys it, but if the pulp of the apple or the potato is pounded with pure glycerine and filtered, the filtrate shows strong oxidase properties Glycerine also extracts some of the chromogen in each case, and hence if diluted with water and exposed to air it darkens rapidly If the glycerine extract is concentrated by soaking cubes of material in glycerine, and then pounding up with a little fresh glycerine, it is obtained as a clear yellowish

liquid which may remain colourless and may show a strong power of decomposing H_2O_2 and turning guaiacum blue for a month or more at 12–14° C. The glycerine extract of the apple if half diluted with water turns brown, and shows faint oxidase reactions after 10 days and none after 15 days. The process of browning may weaken the oxidase in the same way that it is weakened, and finally destroyed, by decomposing an excess of hydrogen peroxide.

The addition of a little of the glycerine extract to the sap from potato cubes just killed by heat causes the production of a purplish-brown colour, whereas the same amount of extract added to pure water produces no distinct change of colour.

When pulp is pounded with glycerine filtering is difficult and prolonged. The best mode of obtaining the oxidase extract is by cutting the pulp into minute cubes or thin slices, and placing these in glycerine for five minutes, then pouring off the now diluted glycerine and replacing by fresh glycerine. In this they may soak for 1–2 days. The glycerine can then be strained off, and, if not diluted with water, keeps without discolouring.

If potato pulp is left in contact with glycerine it discolours on the exposed surface in a few days, but, if well covered by glycerine, remains uncoloured. Pounded pulp repeatedly extracted with an excess of glycerine for three weeks and thoroughly washed with water remains colourless on exposure to air, after the addition of fresh glycerine extract. Hence the glycerine and water can extract the whole of the chromogen from the pulp.

Nevertheless, the oxidase appears to cling with some tenacity to proteids of the cell. Thus pounded potato pulp was allowed to brown, and then washed till quite colourless, and the washing continued for an hour. On testing the pulp it still decomposed hydrogen peroxide energetically and gave oxidase reactions, while another portion remained colourless after the addition of fresh glycerine extract. Evidently the chromogen is easily removed by washing, but not the oxidase. Nevertheless, the latter is soluble in water, for the clear filtrate from pulp pounded with water shows distinct, though not very strong, oxidase reactions.

The glycerine extract of apple pulp shows feebler oxidase properties than that of the potato, and, when diluted, slowly turns reddish-brown on exposure to air.

A simple mode of rapidly obtaining an extract of potato oxidase free, or nearly free, from the chromogen is to pound up to a paste, wash with water, remove the pulp with a strainer, leaving the starch behind. Squeeze out the excess of water, pound up with fresh water, settle and

decant and filter the clear liquid In this way a strong watery extract can be obtained suitable for immediate use, and practically free from the chromogen The oxidases of all the plants examined appear to be soluble in water, or, at least, to pass through a filter paper By half saturation with alcohol they can be precipitated, possibly clinging to precipitated proteids, but with excess of alcohol are attenuated and finally destroyed

The Distribution of the Oxidase in the Cell

Rapidly expressed and filtered apple sap does not decompose hydrogen peroxide, and gives no blue with guaiacum Later filtrates, when the odd pulp cells on the filter paper become brownish, show a very feeble decomposition of H_2O_2, and give a faint blue with guaiacum on long standing

If fragments of fresh pulp crushed between filter paper till dry are added to colourless apple sap, the latter only becomes brownish-yellow after three days, and the former browns distinctly, whereas in water it remains practically colourless.

Apparently, therefore, the oxidase is in the protoplasm, and not in the cell, sap and the browning is more readily produced when the tannic acid is inside the cell than when it is outside

In the case of the potato the expressed sap, however obtained, rapidly discolours in air, and contains both oxidase and chromogen If, however, slices of fresh potato are immersed in colourless apple sap (filtered and boiled), they slowly turn deep brown The brown colour is on the surface layers, and is mainly in the protoplasm, which darkens strongly, and the cell-walls slightly, on adding ferric chloride The tannic acid of the apple sap and the oxidase of the potato meet mainly in the protoplasm of the latter, and the sap outside is only slightly discoloured, and, if plenty of potato is used, contains much less tannic acid Potato oxidase will therefore oxidise apple tannin, but much more slowly than apple oxidase *in situ*, and it appears to be located in the protoplasm of the potato cells. Possibly the potato oxidase may be aided by the less soluble phosphates retained by the potato cells, or may work better in a less acid medium * At least, if the apple sap is nearly neutralised by the addition of dilute ammonia, the browning of the potato slices in apple sap is slightly accelerated, and these become very dark or black on the addition of ferric chloride On the other hand, if an excess of apple sap is used, the browned potato pulp loses its oxidase properties, and, as the liquid gains none, the oxidase has evidently been destroyed.

* According to Hunger ('Bull. d D. Bot. Gesell,' vol. 19, p. 374 (1901)), tannins and glucose often mask the presence of an oxidase or prevent its action. This certainly does not apply in the case of the apple.

Colour and Oxidase Action

Owing to their striking character most attention has been directed to those oxidase reactions which are accompanied by a production of colour The parsnip and carrot have fairly strong oxidases in their cortex, phloem and cambium, but the former has no chromogen, the latter none oxidising further on death The most important oxidase reactions are in fact probably those unaccompanied by any colour change, and in some cases coloured bodies may be rendered colourless by oxidase action

Thus a living slice of potato stained with a watery solution of gentian violet becomes slowly paler when kept moist in air The extracted oxidase of potato slowly partially decolorises gentian violet, and the action is hastened by the addition of small quantities of H_2O_2 Gentian violet may be heated with H_2O_2 without being decolorised but if a drop of a mixture of $CuSO_4$ and sodium or potassium chloride is added to the hot liquid, the latter rapidly becomes colourless

Copper sulphate alone is much less effective Similar results are given by eosin, indigo carmine* and methyl blue (pale purplish) but at temperatures below 50° C, which is the highest to which organic oxidases can be raised with safety, the reductions by the metallic oxidase are very much slower than at 95° C to 100° C Further, in the case of organic oxidases, dilute solutions must be used so as to avoid poisoning the oxidase Even then only partial decolorisation is shown and this is often difficult to distinguish from effects due to absorption Using a strongly oxidase diastase a faint decolorisation was shown in the presence of hydrogen peroxide with dilute solutions of methyl blue and eosin but none with gentian violet or indigo blue with or without peroxide of hydrogen

Since copper sulphate and salt can decolorise indigo carmine, an oxidase can also act as a reducing agent in the presence of an excess of hydrogen peroxide, particularly at high temperatures

The Destruction of Oxidase by Heat.

Although oxidases are not necessarily proteins, cell oxidases seem to adhere closely to proteins, and it is possible that it is the coagulation of the latter by heat that renders the oxidases inactive Their destruction by absolute alcohol might arise in the same way

The glycerine extracts of both apple and potato develop coagulated particles on boiling, at the same time that they lose their oxidase properties.

* Prolonged boiling with excess of H_2O_2 partially reduces indigo carmine to a brown or greenish-brown liquid

If a dilute solution of copper sulphate and salt is mixed thoroughly with an excess of egg albumin, a greenish-white precipitate is formed, but the liquid will still give a strong blue with guaiacum. After boiling, the filtrate gives no blue with guaiacum, and the coagulum gives a greenish colour only Boiling with coagulable proteid would apparently in this case practically destroy the "oxidase" reactions of copper sulphate and salt, and would entirely remove the oxidase from its solution in water

Where, however, a cell oxidase is a metallic salt not combined with or associated with proteins, the oxidase properties may be retained after boiling Instances of these are already known,* and where boiling removes the direct oxidase action of an extract but this returns slowly after cooling, this may be due to the conversion of a "per" salt into a "proto" salt or *vice versâ* Further, a solution of potassium ferrocyanide on standing in light develops traces of ferricyanide and then becomes able to give a direct blue with guaiacum

The Resistance of Oxidases to Drying and Keeping

According to Moore and Whitley (*l c*), apple and carrot gratings dried at 45° C for eight and three days respectively lost all their oxidase Potato pulp was ground up, the excess moisture pressed off and the pulp spread in thin layers to dry in air at 15° C This formed a grey powder when ground It decomposed H_2O_2 moderately actively, mixed with water gave a very faint blue with guaiacum, with ursol tartrate and H_2O_2 the liquid darkened slowly to brown and ultimately purplish Drying makes the oxidase cling firmly to the proteids of the pulp These properties were shown by the powder even when three months old Similarly mere drying did not destroy the oxidase in apple pulp from which the sap had been pressed out before pounding and drying, and the properties were retained uninjured for over three weeks in the dry condition Moore and Whitley's results may have been due to the non-removal of the sap or to the higher temperature used

Even in solution oxidases may retain their properties for a long time. The glycerine extract of potato oxidase, covered, but in contact with air, darkened slowly at 12–15° C, gave after two months a distinct reaction with guaiacum, a slow change through brown to purple with guaiacum, a slow change through brown with ursol tartrate, and a moderately active decomposition of H_2O_2. At three months it gave no blue with guaiacum alone, a faint blue with guaiacum and salt, stronger with guaiacum and H_2O_2, and very slow browning with salt and ursol tartrate, stronger with H_2O_2. At four months it gave a faint blue with guaiacum and H_2O_2 and a moderately active decomposition of

* See Lafar, 'Technische Mycologie,' vol 1, p 675 (1907).

H_2O_2, but no other oxidase reaction At five months it produced a weak decomposition of H_2O_2 but no other oxidase reaction At the eighth month the decomposition of H_2O_2 became practically imperceptible. In this case, by gradual attenuation, the same oxidase became first a "peroxidase" and finally a pure "katalase"

Paraphenylenediamine Test for Oxidase

As is well known, this substance forms an exceedingly sensitive test for oxidases, and goes through a remarkable series of colour changes under their action The full series of colour changes is green, then blue, then brown, then violet, darkening, and in strong solutions forming a black precipitate, but according to circumstances, or if the oxidase action is very intense or very feeble, one or more of these changes may be omitted or modified The chief objections to the reagent are the readiness with which decomposition or oxidation takes place naturally and its excessive sensitivity Grues* recommends the use of the tartrate of paraphenylenediamine (ursol tartrate) This dissolves readily in water, and a pinch of the dry salt can be dissolved in water for each test Any colour change in the clear solution is readily perceptible and there is no alcohol present to interfere with the reaction Further the dry tartrate keeps indefinitely. It is, however, not so sensitive and responds more slowly. On the other hand, it will often give a full colour series, where the alcoholic solution of paraphenylenediamine gives a single colour change only, which, when slow, may be confused with its natural slow darkening on exposure to air

Neither alcoholic paraphenylenediamine nor the tartrate respond to all oxidising agents. Thus nitric acid appears if anything to exercise a reducing rather than an oxidising action It does not produce any colour sequence, and if a little dilute nitric acid is added to potato pulp turned green or blue by ursol tartrate and peroxide of hydrogen, the pulp immediately becomes pale in colour

The reactions with those metallic salts capable of turning guaiacum blue are of interest.

Silver nitrate forms a grey precipitate, slowly darkening, with alcoholic paraphenylenediamine, but in the presence of hydrogen peroxide the colour sequence, green, brown, ruby, violet is given, and the same is given with silver nitrate and ursol tartrate, whereas in the presence of hydrogen peroxide the change is from green to brown only Ferric chloride gives the full colour sequence (green, brown, violet, or purple) with alcoholic paraphenylenediamine,

* 'Biologie der Enzyme,' 1912.

but in the presence of hydrogen peroxide gives a reddish-brown at once. With the watery solution of the tartrate the colour sequence is also given. The presence of free nitric, sulphuric, hydrochloric, citric, or tartaric acids prevents or delays the production of an oxidation colour sequence with ferric chloride, but this takes place readily in the presence of 10 per cent oxalic acid With soluble copper salts (sulphate, acetate, chloride), alcoholic paraphenylenediamine darkens directly without showing any colour sequence, but if the solutions are dilute, and salt and hydrogen peroxide are present, a partial colour sequence from brown to violet or purple is shown

With the watery solution of ursol tartrate no colour change is given with copper acetate, sulphate, or chloride in the presence or absence of sodium chloride With the sulphate and acetate an apparent colour sequence of green to brown is given on the addition of hydrogen peroxide, but this is partly due to the fact that the peroxide gives a greenish colour with copper, and ursol tartrate slowly browns in the presence of hydrogen peroxide With copper chloride, however, a violet or purple tinge ultimately appears, and a full colour sequence (green, brown, violet, or purple) is given with copper sulphate and copper acetate in the presence of salt and hydrogen peroxide. If, however, the solutions are very dilute the colour change is slow, and is direct to brown

Gruss* suggests that the direct oxidation to brown is due to molecular oxygen, and that the colour sequence is the result of the action of atomic oxygen The data given above, however, yield no support to this view The colours produced seem to depend to some extent upon the relative degrees of dilution and intensity of action Colour may be intramolecular or extramolecular, *i e* due to the absorption or modification of light rays at the surfaces of molecules or of molecular aggregates In the latter case if the peculiar aggregation is broken up when the material is in solution the colour may disappear or be modified It is quite possible that the colour sequence with paraphenylenediamine is the result of temporary molecular aggregations during the process of oxidation which react differently to light rays, and whose production depends more upon relative mass action than upon any other factor, this determining molecular aggregations of material in various stages of oxidation

Ursol Tartrate Test for Lignin

This delicate and striking reaction is best shown with boiled or dead tissues by placing them in the watery solution It is a reaction comparable with the phloroglucin test, and is shown in the absence of free oxygen, acid,

* *Loc cit*, p. 11

or light The colour given is brown or brownish red It picks out the wood vessels in a slice of boiled potato or carrot in brownish red without affecting the other tissues The bundles or vascular network on the inner surface of orange or lemon peel are coloured bright red on a white ground, looking like blood vessels injected with carmine Conifer wood or a match also colours bright red As a direct test, it is simpler to apply than any other lignin test, and the colour is confined to the walls of the vessels or tracheides In testing tissues for oxidase this reaction must be borne in mind

The Action of Paraphenylenediamine on the Oxidases of Apple Sap and Potato

Apple pulp turns violet, in a few minutes rapidly darkening to blue, with alcoholic paraphenylenediamine in the absence as well as in the presence of peroxide of hydrogen In the former case the blue colour remains permanent for an indefinite length of time, whereas in the presence of peroxide of hydrogen the oxidation is ultimately completed to a dark brown or black

Potato pulp remains colourless with alcoholic paraphenylenediamine for one or more hours, but on long standing the liquid acquires a brown colour, tinged with violet, and the pulp a weak but distinct violet tinge In the presence of peroxide of hydrogen a violet colour is rapidly produced, but changes to brown in the presence of an excess of peroxide of hydrogen.

Using a watery solution of ursol tartrate, apple pulp develops a violet or blue colour in the absence and presence of peroxide of hydrogen, appearing first in the veins and persisting for a long time With potato pulp and in the presence of peroxide of hydrogen a green colour is shown passing through blue rapidly to a slate colour In the absence of peroxide of hydrogen potato pulp remains colourless, gradually acquiring a slight brown colour in two days, but with no signs of any colour sequence, and the brown is hardly stronger than that produced in pieces of boiled egg albumin used as a control. In all cases no colour sequences were produced by boiled apple or potato pulp. In needing hydrogen peroxide to produce a colour sequence with ursol tartrate, potato oxidase therefore resembles copper sulphate and salt, and, similarly, both the vegetable oxidase and the metallic oxidase give a blue colour with guaiacum in the absence of hydrogen peroxide. That is they are oxidases to guaiacum, "peroxidases" to ursol tartrate

On the other hand, apple oxidase resembles ferric chloride in its ability to produce oxidase colour changes with both ursol tartrate and guaiacum in the absence of hydrogen peroxide

The Potassium Iodide and Starch Test for Oxidase in Living Tissues

Moore and Whitley consider that where a plant extract gives a blue with guaiacum without the addition of hydrogen peroxide being necessary, this is due to the production of peroxides by the dying protoplasm during extraction or to their presence in the guaiacum solution. If H_2O_2 is added to a solution of potassium iodide, iodine is liberated and gives the usual blue with starch On applying potassium iodide to the freshly cut surface of a potato a blue is also slowly formed, as well as with the cut surface of an apple or carrot smeared with starch Bach and Chodat* consider this to prove that the living cells develop peroxides If, however, the material is pounded to a fine pulp and potassium iodide applied to the surface no liberation of iodine takes place, and yet in freshly pounded pulp any peroxides produced by drying cells should be more abundant than in a freshly cut surface Further, the pounded pulp gives a strong blue with guaiacum without the addition of hydrogen peroxide being necessary With strong potassium iodide, pounded pulp browns, and the starch grains swell but remain uncoloured, although staining readily when free iodine or when hydrogen peroxide is added As a matter of fact the liberation of iodine from potassium iodide appears to be due to the oxidase present in the tissues used If a slice is boiled and a fresh surface cut no liberation of iodine is shown Actual tests showed that slices soaked in hydrogen peroxide contained some of the latter undecomposed after again boiling

Certain metallic oxidases such as ferric chloride, black oxide of manganese and potassium ferricyanide will also liberate iodine in a solution of potassium iodide Hydriodic acid is a substance which readily undergoes oxidation with a production of free iodine, and dilute hydrochloric acid liberates free iodine at the surface of a solution of potassium iodide, giving a blue colour in the presence of starch. Bach and Chodat† have shown that the oxidases in the sap of plants can decompose hydriodic acid, although Aso‡ considers this action to be due to the presence of nitrates or nitric acid The solution of potassium iodide we may suppose to contain in addition to ions and undissociated molecules of KI also KHO and HI The latter would be liable to oxidation by organic oxidases when applied on one side of a semipermeable membrane. The action is naturally favoured by the presence of free acid, and is only shown by tissues rich in oxidase. The apple, potato and carrot, which are all acid, give the change readily and the iodine is liberated first over the parts rich in

* 'Ber d. D Chem Gesell,' vol 35, p 2464 and p. 3943 (1902).
† 'Ber d D Chem Gesell,' vol. 37, p. 36 (1904)
‡ 'Bull. Coll Agric,' Tokio, vol. 5, p. 481 (1903).

oxidase, such as the phloem and cortex of the carrot, the veins of the apple and potato A potato kept until the tuber was watery but still acid, and in which the oxidase had nearly disappeared, showed no power of liberating iodine from potassium iodide

The cut surface of the parsnip is neutral or feebly alkaline and, although rich in oxidase, a cut surface shows only a feeble power of liberating iodine from potassium iodide over the phloem ring and outer cortex after many hours' exposure to air The acid pulp of the orange and lemon, which contains no oxidases, is unable to produce any liberation of iodine, nor does the wood cylinder of the carrot, which is usually more acid than the cortex but contains hardly any oxidase

This action is evidently due to the oxidase and not to the free acid The extracted oxidase, however, like pounded pulp is unable to produce any liberation of free iodine from potassium when tested in the usual way Possibly this is because any iodine liberated would at once attack and destroy the plant oxidase where this was in immediate contact with potassium iodide Free iodine does actually destroy potato oxidase Hence to produce any progressive liberation of iodine sufficient to stain the starch the oxidase and potassium iodide would need to be separated by a semi-permeable or colloidal membrane, such as is formed by the cell walls on the cut surface

If pounded potato pulp or filter paper pulp saturated with a glycerine extract of oxidase is covered by a layer of gelatine containing starch or of starch paste, and a little potassium iodide poured on top when the colloid layer has set, after one day a more or less prominent violet line appears on or close to the pulp Apparently the oxidase is only able to liberate iodine from potassium iodide when the latter diffuses slowly to it, and this is possibly a question of relative mass action and osmotic separation.

In any case the liberation of iodine from potassium iodide on the cut surface of a living tissue can be used as a confirmatory test for the presence of an oxidase It does not indicate the presence of hydrogen peroxide or of any special "iodoxidase"

The Influence of Anæsthetics on Oxidase Action

Ether—Small cubes of potato soaked in saturated ether water for a day and then exposed to air darkened distinctly Pulp triturated with ether darkened slightly, and gave strong oxidase reactions and decomposed H_2O_2. The clear ether extract had no oxidase properties. Apple pulp pounded with excess of ether turns a deep brown, but a little more slowly than in the absence of ether The ether extract is yellow, not owing to tannin but

to etiolin The pulp gives strong oxidase reactions, but only decomposes H_2O_2 feebly or not at all If allowed to dry in the air the oxidase reactions are feebler, but the decomposition of H_2O_2 a little more active If the potato pulp ground up with ether is left in contact with it and tested at hourly intervals, it ceases to give distinct oxidase reactions in the following order —Ursol tartrate and H_2O_2, guaiacum, ursol and H_2O_2, guaiacum and H_2O_2, decomposition of H_2O_2 Hence a substance which is at first a "peroxidase," an oxidase and a "katalase," as it is attenuated, becomes a "peroxidase" and "katalase," and finally a "katalase" only

Chloroform.—Apple pulp pounded with an excess of chloroform turns a yellowish-brown, deepening slowly on exposure to air The pulp does not decompose H_2O_2, it gives feeble or doubtful oxidase reactions, which in the case of guaiacum are rendered more distinct by the addition of H_2O_2, but not in those of ursol or its tartrate If the chloroformed pulp is dried in air and powdered up, it regains a weak power of decomposing H_2O_2, and shows stronger but still feeble oxidase reactions Potato pulp triturated thoroughly with excess of chloroform, after the latter had been allowed to evaporate, gave no oxidase reactions with ursol or with the tartrate and H_2O_2, a pale blue with guaiacum on standing, given at once in the presence of H_2O_2, and produced a very feeble decomposition of H_2O_2. The chloroform apparently attenuates or retards oxidase action much more than ether does

Neither chloroform nor ether inhibits the action of metallic oxidases such as copper sulphate and salt, ferric chloride, black oxide of manganese, potassium permanganate, or potassium ferricyanide, but in certain cases chloroform retards or inhibits the decomposition of hydrogen peroxide Thus if a mixture of copper sulphate and salt is shaken up with an excess of chloroform a temporary precipitation film like an exaggerated surface tension film forms on the surface of the chloroform, and on adding H_2O_2 an occasional large bubble may form beneath this film, lifting it up like a skin, but in the liquid above no decomposition of the H_2O_2 takes place If, however, the chloroform is removed by evaporation or the liquid warmed to start the decomposition it continues indefinitely Chloroform itself does not decompose H_2O_2, and saturation with ether slightly lessens the decomposition without arresting it. Similar results were given with ferric chloride, except that the action of the ether is stronger, and if the ether or chloroform is removed by boiling the liquid becomes reddish-brown and loses the power of decomposing H_2O_2, whereas if removed by evaporation at a low temperature the power of decomposing fresh hydrogen peroxide is regained Chloroform added to potassium ferrocyanide and H_2O_2 merely changes a rapid stream of small bubbles into a slow stream of occasional larger

bubbles, and still less influence was exercised upon the decomposition produced by potassium permanganate and black oxide of manganese, although in the latter case some remarkable surface tension effects were exercised

Hydrogen peroxide is readily soluble in ether, which will in fact remove it from a watery solution It is only sparingly soluble in, chloroform, for, although the chloroform solution will not give any blue with chromic acid, it gives a feeble reaction with a watery solution of starch and potassium iodide or with ferrous sulphate Chloroform prevents the ether-chromic acid reaction for hydrogen peroxide being given but not by destroying the hydrogen peroxide In fact the hydrogen peroxide can be shaken with chloroform and the latter then boiled off without the former being destroyed The retarding action on peroxide decomposition produced by ether might depend upon whether the "katalase" salt dissolves in it as well as in water, since otherwise the hydrogen peroxide might be removed from katalase action except at the contact surface In the case of chloroform any bubbles produced form mainly below the surface tension film, although both hydrogen peroxide and the katalase salt may be present in abundance in the liquid above The chloroform apparently acts as an "anæsthetic" to "katalase" chemical action

The Oxidases of the Lemon and Orange

According to Moore and Whitley there are no "peroxidases" in the pulp or rind of these fruits This is hardly the case, as no allowance was made for the effect of the acid in the pulp or of the oils in the skin Quarters of the pulp were squeezed dry in a press between blotting paper, and collected until a sufficiency of clean material free from acid was obtained This gave no reaction with guaiacum alone and none with ursol tartrate except that the fragments of the tracheæ coloured brownish-red On adding a drop of peroxide of hydrogen a pale but distinct blue was given with guaiacum and a slow change to violet with ursol tartrate The pounded pulp does not decompose hydrogen peroxide appreciably On dissecting out the vascular bundles and applying ursol tartrate and peroxide of hydrogen, all the veins right down to the stalks of the endocarpal hairs turned green, then brown, then violet, but no other parts They also showed a feeble power of decomposing H_2O_2 After soaking in orange or lemon juice for some hours or after boiling no oxidase reaction was given but the walls of the tracheæ gave a bright red lignin reaction, making the bundles look like blood-vessels injected with carmine

The Oxidase of the Carrot

According to Moore and Whitley the "peroxidase" of the carrot is most abundant in the protoxylem They were either misled by the lignin reaction or mistook the central wood cylinder of the carrot for pith. With both guaiacum and ursol tartrate in the presence of H_2O_2 the oxidase reaction is given first in the cambium, cambium segments and phloem The central wood cylinder is the last part to show any true oxidase reaction All parts decompose H_2O_2, and in ursol tartrate alone a green colour slowly appears along the line of the cambium, while the vessels in the wood within colour reddish-brown The latter colour appears in a boiled section but not the former We are evidently dealing with a somewhat weak oxidase, most abundant in the cambium and phloem, next in the outer cortex and least of all in the central wood cylinder

According to Moore and Whitley the cut surface of a carrot rapidly develops peroxides and will then give a blue with guaiacum without any addition of H_2O_2 It is difficult to see how this explanation can apply to a tissue like that of a carrot which rapidly decomposes peroxides, or at least peroxide of hydrogen, or to a section of carrot immersed in a large quantity of a watery solution of ursol tartrate, in which the reaction is slowly given by the uncut cells beneath the surface, and where any peroxides formed in the uninjured cells would be washed away Actual tests failed to detect any peroxide of hydrogen in living or dead carrot tissue

The Oxidase of Red Beetroot.

In spite of its red colour the expressed sap of the beetroot shows a strong reaction with guaiacum, but is difficult to use with other oxidants. Hence the sap was squeezed out, the pulp washed, the excess of water squeezed out, and the residue pounded with glycerine, the first portion of which was thrown away In this way a pale pink strongly active oxidase was obtained, which closely resembled potato oxidase. It reacted to guaiacum and tyrosin in the absence, but to ursol tartrate only in the presence, of hydrogen peroxide It has no action on tannic acid by itself and only a feeble one in the presence of sodium phosphate It has a weaker power of decomposing hydrogen peroxide than potato oxidase and the peroxide appears to inhibit its action on pyrogallol, but acts as a sensitiser in the case of ursol tartrate.

The pounded pulp reacts strongly and rapidly to ursol tartrate in the presence of hydrogen peroxide but only slowly and faintly or not at all in its absence. Neither the pulp nor the expressed sap appears to contain any perceptible amount of chromogen capable of oxidation.

According to Bertrand* the sugar-beet contains an oxidase capable of oxidising tyrosin which he terms "tyrosinase," and this, according to Gonnermann,† oxidases tyrosin to homogentisinic acid, which darkens rapidly by direct oxidation to red, brown, or black. In the red beet the amount of tyrosin present appears to be too small to appreciably affect the neutral red colour It may undergo oxidation, while the plant is living, and hence be unable to accumulate

The Oxidase of the Parsnip

The parsnip differs from all the other vegetables used, in that a cut surface is neutral or faintly alkaline instead of acid, and it resembles the carrot in containing no chromogen oxidising on death. Neither carrot nor parsnip oxidase will directly brown boiled potato or apple pulp, but if a little sodium phosphate and H_2O_2 is added, they will cause tannic acid, apple pulp and apple juice to brown distinctly and with fair rapidity

In both carrot and parsnip the oxidase is mainly present in the phloem and outer cortex, and that of the carrot appears to be a little more abundant Hence of similarly prepared watery or glycerine extracts the former is a little more active than the latter

	Beetroot oxidase	Carrot oxidase	Parsnip oxidase
Guaiacum	+ + +	+ +	+ +
Do $+ H_2O_2$	+ + +	+ + +	+ + +
Ursol tartrate			
Do $+ H_2O_2$	+	+	+
Pyrogallol	+ +	+	
Do $+ H_2O_2$	+	+ + +	+ + +
Hydroquinone			
Do $+ H_2O_2$	+	+	+
Gallic acid			
Do $+ {}_2H_2O$	+	+	+ + +
Tannic acid			
Do $+ H_2O_2$			
Tannic acid and sodium phosphate			
Do $+ H_2O_2$		+	+
Tyrosin	+ +		
Do $+ H_2O_2$	+		

Both ursol tartrate and hydroquinone when applied to a cut surface of a carrot or parsnip show signs of oxidation particularly over the phloem ring. This is probably because the oxygen is more concentrated at the surface and also a greater mass action is exercised upon the inwardly diffusing oxidant. No assumption of the production of peroxides in the tissue is necessary to explain the action. The addition of magnesium sulphate or of potassium

* 'Bertrand, 'Compt. Rend.,' vol 122, p. 1215

† Gonnermann, 'Pflüger's Archiv,' vol. 82, p. 289 (1900)

phosphate to carrot or parsnip oxidase causes it to give a faint trace of browning in one day with tannic acid which is not increased by the addition of hydrogen peroxide None of the other salts which could be made up from the ash constituents (excluding iron salts) exerted any sensitising oxidase action The oxidase of the beetroot and potato appear to belong to one class (betase, potatase, dahliase, russulase), those of the carrot and parsnip to another, while the chief peculiarity of apple oxidase, namely, the readiness with which it oxidises tannic acid, appears to be due to the presence of a sensitiser such as potassium phosphate Apple oxidase appears to have some resemblance to a weak form of laccase which is also able to oxidise tannic acid

Summary

Plant oxidases form a class of substances of great importance in plant metabolism, but which are known merely by the reactions they cause, and whose exact nature is quite unknown

According to Bach and Chodat they form three distinct classes of ferments namely —

(1) Oxygenases, substances which absorb molecular oxygen forming peroxides

(2) Peroxidases, which increase the oxidising power of peroxides and can only act in their presence

(3) Katalases, which destroy peroxides with an evolution of oxygen.

It has long been known that certain of the reactions supposed to characterise oxidase ferments could be produced by certain inorganic metallic salts* As the result of the detailed investigation of the oxidase action of various metallic salts of copper, iron, chromium, manganese, lead, etc, upon guaiacum, paraphenylenediamine, hydroquinone, pyrogallol, gallic acid, tannic acid, and tyrosin, the conclusion has been formed that the correspondence between the action of organic and of inorganic oxidases is extremely close It was also found that the oxidase action of a metallic salt varies according to its acid combination, and that in the case of certain salts, such as sodium or potassium ferrocyanide, ferricyanide, phosphate, or chromate, the oxidase action was due to the acid and not to the base. In addition, oxidase action may be accelerated in the presence of sensitisers such as the chlorides or phosphates of sodium or potassium, or retarded or prevented by a variety of antagonisers. The addition of a sensitiser may cause a "peroxidase" to act in the absence of hydrogen peroxide. This applies to both organic and inorganic oxidases, and determinations of the minimal amounts of metallic

* Bertrand, 'Compt. Rend,' vol. 122, p 1032 (1896).

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oxidases required to produce progressive oxidation in the presence of a sensitiser indicate that their action can be considered as closely akin to that of any enzyme H E and E F Armstrong* have shown in a series of valuable papers, and particularly in the hydrolysis of raffinose by acids and enzymes, that a close correspondence exists between the action of organic and inorganic hydrolysing agents The same appears to hold for organic or inorganic oxidases.

In general, oxidases, whether inorganic or organic, may vary from strong to weak The former will cause direct oxidation from the oxygen dissolved in a watery solution The latter will transfer oxygen from labile oxygen compounds such as hydrogen peroxide, or will use dissolved oxygen in the presence of sensitisers such as the chlorides or phosphates of sodium or potassium Various intermediate grades of activity are shown There is no reason for separating oxidases and peroxidases as distinct classes of ferments, and peroxides do not necessarily take part in all oxidase actions, although water does The supposed separation of oxidase and peroxidase by fractional precipitation with alcohol may be merely the result of attenuation

An oxidase may be a "peroxidase" to certain oxidants or may become so when attenuated Metallic oxidases act as ferments in that a small amount may produce considerable oxidation, especially in the presence of sensitisers such as salt with copper sulphate, sodium phosphate with potassium ferricyanide, etc, and in that the oxidase appears to act as an intermediary in the chemical change.

Hydrogen peroxide may influence oxidase action —

(*a*) By providing a supply of labile oxygen.

(*b*) By converting a feeble oxidase into a strong oxidase (ferrous salt into ferric, ferrocyanide into ferricyanide)

(*c*) By acting as a sensitiser to the oxidant substance

(*d*) By acting as an inhibitor or antagoniser in some cases

Various salts may act as sensitisers (sodium and potassium chlorides, bromides, and phosphates) or as inhibitors (barium chloride, sodium fluoride, organic or inorganic acids), and in some cases, with increasing concentration, the action of the former is reversed, while a substance which is a sensitiser with one oxidant may act as a reducing agent with another (copper sulphate and salt on indigo carmine).

Strong metallic poisons will arrest the action of organic oxidases or destroy them (apple, potato, carrot, parsnip) if immediate contact or rapid

* 'Roy Soc. Proc.,' B, vol. 82, p 349 (1910); vol. 80, p. 312 (1908), etc.

penetration is assured Hence the organic oxidases are possibly proteids, with or without oxidase metals, in basic or acid combination

There is no justification for the use of such terms as "peroxidase," "katalase," "œnoxydase," or "tyrosinase," to indicate specific substances, ferments, or groups of ferments The "tyrosinase" of the potato is also a "katalase," a "peroxidase," a "pyrogallase," a "hydroquinonase," and a "paraphenylendiaminase." It is, however, permissible to use such terms as katalase action or peroxidase action, and such names as laccase, russulase, potatase, carrotase, etc, as temporary names to indicate the origin of the substances, whose chemical nature is yet unknown Since, however, their oxidase powers will be only one of many properties, it will never be advisable to name them according to these properties alone, any more than it would be in the case of the metallic oxidases Comparison with metallic oxidases shows that we are not even on safe ground in assuming the existence of specifically distinct classes of plant oxidases, such as phenolases, aminoxidases, and iodoxidases

The chlorides and phosphates of potassium and sodium are able to act as oxidase sensitisers, and thus may influence special oxidations, or respiration in general. It is possible that they may exert a stimulatory or controlling action on plant metabolism, and that the sodium chloride always present in the ash of plants may not be an entirely useless constituent This may explain partly why small doses of salt stimulate the growth of many plants, and why phosphates, in addition to being food substances, may act as stimuli to growth. The stimulating action of many metallic salts on growth may be partly due to their oxidase action

Ursol tartrate turns lignified walls red or reddish-brown This is not an oxidase reaction, but is an admirable test for lignin, especially valuable for demonstrating the wood elements in pulpy tissue

Chloroform strongly, and ether more feebly, retard or inhibit katalase action, but they do not suppress oxidase action After prolonged contact, however, the organic oxidases are slowly attenuated and destroyed

The liberation of iodine from potassium iodide may be used as a test for the presence of oxidases in living tissues, but does not indicate the existence of any power of producing peroxides Dried organic oxidases may retain their properties for three weeks or more, and a glycerine extract for five or more months Where organic oxidases are destroyed by boiling, this is probably the result of proteid coagulation

The oxidases of the beetroot and potato appear to be related to one another, and to be among the strongest plant oxidases, and the nearest analogies to them are perhaps afforded by ferric salts and ferricyanides. If

the special action of apple oxidase on tannic acid is due to the presence of a phosphatic sensitiser, it would be a feebler oxidase of the same type Carrot and parsnip oxidases are a grade feebler, but still react to guaiacum in the absence of a peroxide Malt diastase is still weaker, and papain feebler still, while pepsin may show a weak "peroxidase" reaction with guaiacum, but not any other oxidase action

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The Fixation of Arsenic by the Brain after Intravenous Injections of Salvarsan.

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(Communicated by Prof W Bulloch, F R S Received July 8, 1914)

(From the Bacteriological Laboratory of the London Hospital)

During the period of probation of salvarsan as an anti-syphilitic remedy, a number of toxic phenomena were reported which led to the belief that this drug had particular neurotropic properties, and was therefore to be used with the greatest circumspection These fears were very largely founded upon the well known effect of the related drug atoxyl in producing optic atrophy Subsequent experience has, however, shown that the supposed neurotropic action of salvarsan was due to certain technical errors in its administration

In 1911 we published (1) an observation which combated the view that salvarsan had neurotropic qualities. We submitted the organs of an infant who died after administration of salvarsan to Dr W H Willcox for analysis, and he reported to us that the brain in this case contained no arsenic, although considerable quantities were present in other organs. We then applied the law of Ehrlich, "corpora non agunt nisi fixata," and argued that, since the brain was free from arsenic, salvarsan could have no neurotropic action.

Exactly similar conclusions were arrived at by Ullmann in 1913 (2) In the course of a very extensive investigation upon the distribution of arsenic in the body after salvarsan injections, he made it quite clear that the brain never contained more than traces of arsenic, and "this fact was evidence against the neurotropic action of salvarsan." Similarly, Morel and

Mouriquand (3) found the brain and cord in five animal experiments to be free from arsenic, although other organs contained much They also concluded that salvarsan has no neurotropic effect On the other hand, Mouneyrat (4), after the injection of salvarsan into animals, found arsenic "in very appreciable quantities in the liver, muscles, and brain, distinctly more than the infinitesimal amounts met with in the control animals, which had had the same food but no injection" Thus Mouneyrat considered that salvarsan was "particularly neurotropic" In criticism of this paper it must be stated that the author gives no evidence of having made quantitative estimations, by which he might have discovered that the liver and kidney, for example, contained vastly more arsenic than the brain Further, the fact that he was able to demonstrate arsenic in the brains of normal animals must lead to a certain suspicion of his other results

The observations of Ullmann, Morel and Mouriquand and ourselves were accepted by Ehrlich (5) as showing that salvarsan has no "Vorliebe" for the brain

As a result of subsequent investigations upon the minute anatomy of the cerebral vessels, however, we were not entirely satisfied that the deductions we had drawn were correct

It appeared that absence of arsenic from the brain might be due to two distinct factors Firstly, the arsenic might not be "fixed" by the brain as already suggested, or, secondly, it might not gain access to the brain, owing to some peculiarity of the cerebral vessels In order to test this possibility we conducted experiments *in vitro*, applying neosalvarsan directly to fresh brain substance, and then found that neosalvarsan was in fact "fixed" by the brain

Experiment 1—100 grm of fresh human brain and 100 grm of human liver were minced and placed in two separate glass bottles of 1000 c c capacity These bottles were filled with saline solution, shaken, allowed to stand, and the supernatant fluid removed. The tissue was thus freed from excess of blood, 500 c.c of saline solution, containing 0 15 grm of neosalvarsan, were then added to each, and the bottles were shaken for one hour. At the end of this time the supernatant fluid was removed by decantation and the bottles filled with saline solution eight times, shaking on each occasion and removing the washings by decantation By this method it was hoped that all "unfixed" neosalvarsan would be removed from the tissues.

Estimations of the amount of neosalvarsan in the washed tissue, the supernatant fluid after fixation, and the final washing fluid were then made.

In every case the material to be tested was heated with concentrated nitric

acid and sulphuric acid, the residue extracted with water and tested in the Marsh apparatus The technique employed was that advocated by Chittenden and Donaldson (6) The rings of arsenic obtained in this way were compared with a standard series of rings made from known weights of neosalvarsan, and the amount of arsenic was expressed as grammes of neosalvarsan In no case was any attempt made to obtain accurate quantitative results, and our figures merely indicate roughly whether a particular sample contained much or little neosalvarsan —

		Gramme of neosalvarsan.
Moist brain residue after washing, 100 grm	contained	0 004
Supernatant fluid after fixation with brain, 500 c c	„	0 1
Final washing fluid . .	„	0
Moist liver residue after washing, 100 grm	„	0 015
Supernatant fluid after fixation with liver, 500 c c	„	0 1
Final washing fluid	„	0

From this experiment it appears that brain substance will fix considerable quantities of neosalvarsan *in vitro*

Experiment 2—Repetition of Experiment 1

		Gramme of neosalvarsan.
Moist brain residue after washing, 100 grm	contained	0 01
Supernatant fluid after fixation, 500 c.c	„	0 05
Final washing fluid .	„	0
Moist liver residue after washing, 100 grm	„	0 01
Supernatant fluid after fixation, 500 c c	„	0 05
Final washing fluid	„	0

Experiment 3—Repetition of Experiment 1, but twice the quantity of brain and liver used, viz, 200 grm The results were similar to those obtained in the other experiment, although the brain substance appeared to fix more neosalvarsan than did the liver, thus —

	Gramme of neosalvarsan
Moist brain residue, 200 grm contained	0·02
Moist liver residue, 200 grm „ .	0 004

It may be objected to these experiments, that the arsenic was merely entangled mechanically with the minced tissues and was not chemically fixed; we therefore repeated the experiment after replacing the animal tissues with fragments of Doulton filter (asbestos) and finest vegetable charcoal.

Experiment 4.—50 grm. of broken-up filter candle were placed into 250 c.c. of saline solution and 0·075 grm. of neosalvarsan added. Further technique as in Experiment 1.

Result.—

		Gramme of neosalvarsan
Moist filter residue after washing, 50 grm.	contained	0
Supernatant fluid after fixation, 250 c.c.	"	0·04
Final washing fluid	"	0

Experiment 5.—Repetition of Experiment 4, but with finest Venetian charcoal instead of Doulton filter. Air was removed from the charcoal by boiling and the use of the vacuum pump—0·15 grm. of neosalvarsan were added.

		Gramme of neosalvarsan
Moist charcoal residue after washing, 50 grm.	contained	0·001
Supernatant fluid after fixation, 250 c.c.	"	0·11
Final washing fluid	"	0

Although a certain degree of absorption of the neosalvarsan was observed in the case of charcoal, it was not so great as with liver and brain tissue, and this fact, taken with the absence of fixation by the filter, suggests that the presence of arsenic in the latter after washing was a true combination and not a purely mechanical phenomenon.

The conclusion may therefore be drawn that the absence of arsenic from the brain after intravenous injection of salvarsan is not due to a lack of affinity between the drug and the brain, but to an inability of the drug to penetrate into the proper brain substance.

As is well known, arsenic is not usually found in the cerebrospinal fluid after intravenous injections of salvarsan, and this has been suggested as an explanation of the lack of effect of this drug upon certain syphilitic affections of the brain. On other data, however, we have formed the opinion that the inability of arsenic to penetrate the brain has no relation to its absence from the cerebrospinal fluid, but is due to a peculiarity of the cerebral capillaries. If, however, neosalvarsan is introduced directly into this fluid by lumbar puncture, then, as shown by Wechselmann, marked toxic symptoms appear.

Experiment 6. *To Demonstrate the Toxicity of Neosalvarsan to the Nervous System.*—In a typical experiment two rabbits were used in addition to a control.

0·05 grm. of neosalvarsan were dissolved in 10 c.c. of saline solution, and

of this solution 0·2 c.c. were injected into the cerebrospinal fluid of a rabbit weighing 500 grm., through the posterior occipito-atlantoid membrane. The effect of the injection was to produce convulsions immediately, and death the next day. A second rabbit (800 grm.) received 0·1 c.c. and was found to be paralysed on the following day. As a control, 0·2 c.c. of saline solution was shown to be innocuous. It thus follows that about 0·0005 grm. of neosalvarsan is the toxic dose to a rabbit of 800 grm. weight when injected into the cerebrospinal fluid. It may be assumed that the drug is rapidly absorbed from the cerebrospinal fluid into the brain and is there fixed as in our experiment conducted *in vitro*. Thus salvarsan may be said to be as much "organotropic" to the brain as to the liver, but this effect is not apparent after its therapeutic administration in the ordinary manner.

The inability of salvarsan to penetrate into the brain explains the lack of success which often attends the treatment of syphilitic lesions of the brain, and in particular the parenchymatous varieties (dementia paralytica and tabes dorsalis).

We next considered whether this inability might be due, in some measure, to the rapid fixation of the drug by the liver and kidneys and its removal from the blood.

We have indeed found, experimentally, that the blood is practically free from arsenic two days after an injection, as shown in the following experiment:—

Experiment 7.—Four rabbits were injected intravenously, each with 0·15 grm. of neosalvarsan. They were killed 3, 6, 24, and 72 hours after the injection. Specimens of blood were collected and the animal transfused with saline to remove the blood from the organs. The blood and organs contained the following quantities of neosalvarsan:—

	Rabbit A, 3 hrs.	Rabbit B, 6 hrs.	Rabbit C, 24 hrs.	Rabbit D, 72 hrs.
	Grammes of neosalvarsan			
Brain (*ca.* 10 grm.)	? trace	? trace	0	0
Liver (10 grm.)	0·0005	0·00075	0·0012	0·00025
Blood (25 c.c.)	0·003	0·0006	0·0004	0

We next endeavoured to satisfy the affinity of the liver and kidneys for arsenic by repeated injections of neosalvarsan, in the hope that a sufficient content would be maintained in the blood to allow of penetration into the brain.

Experiment 8.—Three rabbits were injected, each twice daily for four days

with 0 05 grm of neosalvarsan They were killed 12, 36, and 60 hours after the last dose and transfused as before.

The following results were obtained —

	Rabbit A, 12 hrs	Rabbit B, 36 hrs	Rabbit C, 60 hrs
		Grammes of neosalvarsan	
Brain (*ca* 10 grm)	0 ·0001	0 ·00004	0
Liver (10 grm)	0 0005	0 ·00075	0 ·00075
Blood (25 c c)	0 0003	0 ·0001	0

Experiment 9—Repetition of Experiment 8, but all the animals killed 24 hours after the last dose

	Rabbit A	Rabbit B	Rabbit C
		Grammes of neosalvarsan	
Brain (*ca* 10 grm)	0	0	0
Liver (10 grm)	0 00012	0 ·0001	0 0005
Blood (25 c c)	0	0 0001	0

From these experiments it appears that no definite penetration of the drug into the brain can be obtained by a repetition of the injections, although in one case a small quantity was observed 12 hours after the last dose

Similar results were obtained with Prof Ehrlich's new copper-salvarsan combination (k_3)

Experiment 10—Technique as in Experiment 8. The dose of k_3 was 0 02 grm and the animals were killed 24 hours after the last injection

	Rabbit A	Rabbit B
	Grammes of neosalvarsan.	
Brain (*ca* 10 grm)	0	0
Liver (10 grm)	0 00002	0 0005
Blood (25 c c)	0	0

Conclusions

1 After intravenous injections of salvarsan and neosalvarsan in man and animals no arsenic can be found in the brain

2 This phenomenon is not due to a lack of affinity between the brain and the drugs, but to an inability on the part of the drugs to penetrate into the substance of the brain.

3. Fixation of arsenic by the brain occurs as readily as by the liver, as shown by experiments *in vitro* and the toxic effects of intrathecal injections

4 Penetration of neosalvarsan into the brain cannot be obtained even by frequently repeated intravenous injections.

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4 A Mouneyrat, 'Compt Rend de l'Académie des Sciences,' vol 154, p 284 (1914)
5 P Ehrlich, 'Abhandl über Salvarsan,' vol. 3, p 546 (1913)
6 R H Chittenden and H H Donaldson, quoted by A W Blyth in 'Poisons,' p 568, London, 1895

The Production of Anthocyanins and Anthocyanidins.—Part II

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(Communicated by Prof Frederick Keeble, F R S Received July 15, 1914.)

The author of the present paper* and other investigators† have concluded as the result of their investigations that the red pigments obtained as the result of careful reduction of the yellow flavonol derivatives are identical with the natural anthocyans of plants Prof. Willstatter, on the other hand, holds‡ that these artifically produced red pigments are different from the natural anthocyans §

That the point at issue is important may be judged from the facts that, the complete synthesis of flavonol derivatives having been realised by Kostanecki, the production of anthocyans from them as described by the author (*loc. cit*), Watson and Sen,|| and Combes,¶ completes the synthetic production of these compounds Moreover, the production of anthocyan glucosides—anthocyanins

* 'Roy Soc Proc,' B, vol. 87, p. 444 (1914)

† *Cf ibid*, p 446

‡ 'Sitzungsber K Akad Wiss Berlin,' vol 12, p 402 (1914)

§ Since the present paper was forwarded for publication, Wheldale and Bassett, 'J. Genetics,' vol 4, p 103 (1914), have published a criticism of the author's views, in which they also take up a similar attitude.

|| 'Chem Soc Journ,' vol. 105, p 389 (1914)

¶ 'Compt Rend,' vol 157, pp 1002 and 1454.

—from yellow glucosides of the flavonol series by reduction in acid solution as described by the author (*loc. cit.*), is of vital interest to those studying the production of these pigments in plants

Willstätter bases his criticism of the author's conclusions upon the following observations —(1) The red pigments obtained by reduction are unstable, becoming decolorised even in acid solution (2) The decolorised solutions of these pigments do not recover their colour on acidification, even with considerable excess of acid. The anthocyans do not behave in this manner, they are stable in acid solution, and, in neutral solution—with a few exceptions—become decolorised by isomerisation, but the decolorised solution on acidification regains its colour.

The author has carefully examined the red pigments obtained by him, and compared them with cyanin The glucosides and the one non-glucoside examined gave results in every way similar to those given by cyanin and cyanidin respectively, and support the conclusions reached in the author's previous paper (*loc cit*), viz that these pigments are true anthocyanins and anthocyanidins

It is perhaps necessary to point out at this stage that, for the comparisons made by Willstatter and now to be discussed, the anthocyanins—glucosides—give far more reliable results than the anthocyanidins—non-glucosides—which latter compounds are more unstable in solution, even when faintly acid, than the corresponding anthocyanins This fact was first observed when working with cyanin, moreover, the decolorisation by isomerisation and return of colour by the action of acids is far more difficult to carry out satisfactorily with the anthocyanidins, particularly if they are somewhat impure, than with the anthocyanins With the anthocyanins this series of changes is most characteristic and very easily carried out, even when the pigment is in quite a crude and impure condition

As Willstatter appears to have used the non-glucosides only in making his comparisons, the foregoing considerations may perhaps explain the discrepancy between his results and those obtained by the present writer

In every case the red pigments—glucosides—obtained by the author are stable in aqueous or alcoholic acid solution, indeed many of them have remained in open test-tubes for many days without losing their colour In no case has decolorisation under such conditions been observed These pigments become decolorised, it is true, even in acid solution, if reducing or oxidising agents are present, but this is equally true of anthocyanins.

The non-glucosides when in neutral, or very faintly acid, solution are somewhat less stable than the glucosides, but in this they resemble the anthocyanidins

The red acid solutions of the glucosides obtained by the author, when made neutral by shaking with excess of calcium carbonate, pass to violet or red-violet, and rapidly become decolorised. On filtration, and addition of a few drops of acid (HCl used) to the decolorised solution—either soon after decolorisation, or even after standing over night in that condition—they quantitatively regain their red colour, thus behaving as true anthocyanins

The acid solution of the non-glucoside examined, on shaking with excess of calcium carbonate became violet After filtration, the solution on warming, loses the violet colour, but on addition of a few drops of acid and warming the red colour reappears, but not quantitatively A solution of cyanidin yielded exactly parallel results As already mentioned, unless the pigment be pure, or nearly so, the complete recovery of colour is difficult to obtain in the case of the non-glucosides

Besides the examination of the observations of Willstatter, the comparison of the absorption spectra of the pigments obtained by the author with those of cyanin and cyanidin—in acid solution—has been carried out

The pigments all show one characteristic band, resembling in every respect that shown by cyanin and cyanidin chlorides, and agreeing with that described by Willstatter for the characteristic band of the anthocyans in acid solution, as a broad band which becomes flatter towards the violet, and extends over a great portion of the green and blue regions

The author has therefore been able to show that by reduction of flavonol glucosides—the yellow pigments present in many flowers—a series of red pigments may be obtained whose properties agree with those of the anthocyanins A brief summary of the points of agreement may be given

(1) The glucosides remain quantitatively—the monoglucoside from quercitrin is apparently an exception—in dilute (about 2N) sulphuric acid solution when shaken with amyl alcohol, whereas the hydrolysed products pass quantitatively into the alcoholic layer, yielding a red solution which shaken with sodium acetate solution becomes violet or red-violet, the pigment remaining in the alcohol, and their solution on shaking with sodium carbonate solution turns blue or blue-green and at the same time the pigment descends quantitatively to the aqueous layer All these changes are exactly similar to those observed with anthocyanins.

(2) In acid solution—aqueous or alcoholic—they are stable, and their absorption spectra are similar to those of the anthocyanins.

(3) By oxidation—*e g* hydrogen peroxide—their acid solutions may be decolorised. By reduction—*e.g* Zn and HCl—the red colour of their acid solutions is discharged, but reappears on careful addition of hydrogen peroxide. Anthocyanins react in an exactly similar manner

(4) These pigments show the same decolorisation in neutral solution, due to isomerisation, and return of colour by the action of acids as do the anthocyanins

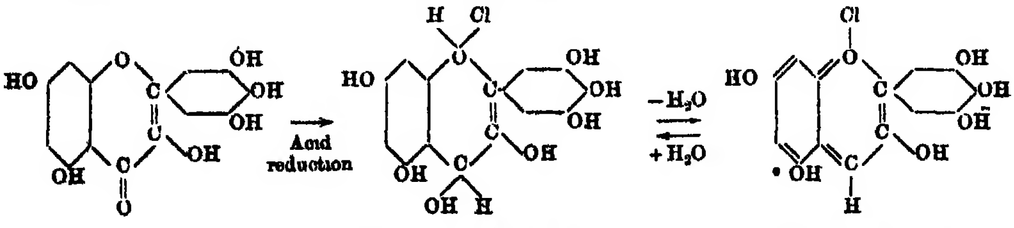
(5) They form red compounds with acids (everything points to these compounds being oxonium salts), are violet or red-violet in neutral solution, and most of them yield blue or blue-green compounds with alkalis, completely resembling in this respect the anthocyanins.

(6) They are very unstable towards alkalis, as are the anthocyanins.

With this evidence may be coupled that of Combes (*loc cit*), who, working with *Ampelopsis hederacea*, obtained a yellow and a red pigment (anthocyan), and by reduction of the yellow obtained the red, and conversely by oxidation the red passed back to the yellow He prepared both of these compounds in crystalline condition and compared their melting points. He did not state whether they were glucosides or no

It seems, therefore, that all available evidence favours the author's conclusions that the red pigments obtained by the reduction of the flavonol derivatives and their glucosides are true anthocyanidins and anthocyanins respectively Prof Willstatter, when lecturing on this subject at University College, London, in May last, expressed the opinion that they are compounds in which the pyrone ring has been opened up at the oxygen linkage Comparison of their properties with those of known compounds of the structure suggested does not lend support to this conjecture, moreover, it would be very difficult to account for their being strong oxonium bases—as they appear to be—if they were of this type, nor could this interpretation supply satisfactory explanations of the change from violet to red on treatment with acids, nor of the fact that the red are more stable than the violet compounds.

It is of interest to note that Willstatter's work supplies important experimental evidence in favour of the scheme



(Myricetin) Flavonol derivative. — Colourless or faintly coloured intermediate product — Anthocyan pigment.

already put forward by the author* to explain the formation of the

* *Loc cit*, p. 449.

anthocyans from flavonol derivatives Willstätter agrees with the author with regard to the structure of the anthocyan molecule, and further, his analytical results (*loc cit*) have shown that the colourless iso-forms are produced by the addition of one molecule of water to the anthocyan molecule, this is in agreement with the above scheme

As flavone derivatives, which also occur in plants, yield somewhat similar red pigments, it seems probable that further examination of the natural anthocyans may yield compounds related to this class of pigment Again, no natural anthocyan pigment has as yet been examined in which the hydroxy-groups in the phenyl group attached to the benzo-pyran residue are in the meta-position to one another As morin, a flavonol of this type, occurs naturally, and on reduction yields a red pigment, it is possible that natural anthocyans of this type may be among the fruits of further research on these pigments It is quite likely that such compounds would differ slightly from those at present investigated

Apart from the anthocyan question, the observations recorded appear to be of interest, in that, as in every case where the author obtained an anthocyan by reduction of the flavone or flavonol present in flower extracts, he was able to show that the pigment was an anthocyanin—glucoside—it would follow that in each case the flavone or flavonol derivative in the plant must have been present in the form of a glucoside

Experimental

The methods of obtaining anthocyanins and anthocyanidins from flavonol derivatives by reduction have already been described by the author (*loc. cit*) The method which gave most satisfactory results, viz.. reducing the compound dissolved in a mixture of 5 vols. absolute alcohol and 1 vol concentrated HCl by means of magnesium (ribbon or turnings), was then used only when pure or nearly pure yellow compounds were obtainable. By slight adaptation, however, it has been found possible to use it with advantage in many cases when dealing with plant extracts It is only necessary to add to the extract of the yellow pigment in 2N HCl its own volume of concentrated HCl and then to the whole 4–5 times its volume of absolute alcohol, for the reduction with magnesium to proceed readily. In some cases it has been found advantageous to add a drop or two of concentrated HCl from time to time A disadvantage of this method is the somewhat considerable dilution which results

Aqueous solutions of the red pigments produced may be obtained from the aqueous alcoholic solutions above mentioned, by the addition of much ether, and if necessary, a small quantity of dilute acid. The red pigments are left

in the aqueous acid layer, provided that sufficient ether is added. The ethereal layer contains much of the unchanged flavonol derivatives and these may be more completely removed from the aqueous acid solution by repeated extraction with ether.

In the preparation of extracts from flowers, dried and powdered petals are more convenient to use than fresh flowers.

With the red pigments—all of which were shown by the amyl-alcohol reaction to be glucosides—obtained by reduction from extracts of the following flowers, viz. daffodil, primrose, viola (yellow) and wallflower (lemon-yellow), the following experiments were carried out, viz.:—

(1) The stability of the pigments in aqueous or alcoholic acid solutions was examined. In every case they were stable, no decolorisation was observed, even after standing for many days.

(2) An acid (HCl) solution (aqueous or alcoholic) of the pigment was separated into two portions: (*a*) kept for comparison; (*b*) shaken with excess of powdered calcium carbonate, whereby all excess of acid was removed, colour passed from red to violet, solution then became decolorised—all traces of red or violet disappeared, solution being a faint brownish yellow. The red colour was restored quantitatively on acidification by 2–3 drops of concentrated HCl (concentrated used to prevent unnecessary dilution) whether the acidification took place immediately after decolorisation or after allowing the decolorised solution to stand over night.

(3) The absorption spectra were examined, with results already mentioned.

Thanks to the generosity of Prof. A. G. Perkin, F.R.S., in placing at his disposal a quantity of crude rutin—a pigment consisting of quercetin combined with a disaccharose residue, and identical with viola-quercitrin*—the author was enabled to carry out similar experiments using the pigment derived by reduction from this flavonol disaccharide of known constitution. The red pigment remained almost quantitatively in aqueous sulphuric acid (about 2N) when shaken with amyl alcohol, only traces passing into the alcohol. When hydrolysed by boiling with hydrochloric acid (about 20 per cent.) it yielded a sparingly soluble non-glucoside, which, when shaken in dilute sulphuric acid solution with amyl alcohol, passed quantitatively into the alcoholic layer, yielding a fine red solution. On shaking the solution with sodium acetate, the colour changed to violet, remaining, however, in the alcoholic layer. On shaking with sodium carbonate solution it became blue-green and passed quantitatively to the aqueous layer.

The series of observations (1, 2, and 3) as above was repeated with the pigment obtained from rutin, and in every way similar results obtained.

* *Cf.* A. G. Perkin, 'Chem. Soc. Journ.,' 1910, p. 1776.

From the specimen of rutin supplied by Prof Perkin, a sample of the crystalline pigment was prepared according to his instructions, and from this also the red pigment obtained and subjected to similar treatment. It yielded exactly similar results.

This case is of particular interest, for, if the disaccharose is attached to the flavonol molecule in the correct position, the pigment produced should be identical with cyanin

For comparison with the above glucoside pigments, the same series of experiments was carried out with cyanin chloride, and gave exactly similar results

From the red pigment obtained by reduction of quercitrin (Kahlbaum) or from rutin, the non-glucoside pigment was obtained by hydrolysis, collected, dissolved in a mixture of equal parts by volume of ethyl alcohol (absolute) and 2N hydrochloric acid (aqueous), and of the solution obtained one part was retained for comparison, the other shaken with excess of finely powdered calcium carbonate to remove acid The colour changed to violet, and after filtration and warming became decolorised—lost all violet colour, only faint brownish-yellow colour remaining On acidification with 2–3 drops of concentrated hydrochloric acid and warming, the red colour returned, but not quantitatively A comparative experiment using cyanidin chloride gave exactly similar results

In solution—even faintly acid—this non-glucoside pigment is not so stable as are the glucosides above mentioned, thus, after standing for a week the colour is considerably diminished, and this diminution is due to destruction Cyanidin, however, behaves similarly, if somewhat impure

The cyanin and cyanidin chlorides used were obtained from a specimen of cornflower pigment containing about 10 per cent of pure pigment

The author desires to express his thanks to Prof A. G Perkin, F R S., for supplying him with the sample of rutin used in some of the above experiments

Observations on the Life-Cycle of a New Flagellate, Helkesimastix* fæcicola, *n.g., n.sp.: Together with Remarks on the Question of Syngamy in the Trypanosomes.*

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(Communicated by Prof. S. J. Hickson, F.R.S. Received July 31, 1913.)

[Plates 13 and 14.]

This new flagellate occurs frequently in goat-dung; we have found it also in sheep-dung. It is a "passenger," being carried passively through the alimentary tract, in an encysted condition. When the dung is moistened with water—probably, in nature, when it is deposited on damp grass or earth—the flagellate emerges from its cyst and goes through its life-cycle, ultimately encysting again. The cysts are doubtless swallowed by the goat with its fodder. We have cultivated *Helkesimastix* under various conditions, which will be described in our full account later. In order to obtain this form in large numbers and study its life-history without any fear of being misled by stages in the life-cycle of other flagellates, we succeeded in isolating it from the other forms occurring in simple dung-cultures and cultivating it on agar-media, on which it multiplies rapidly. The medium which we have used principally is weak Lemco-agar, *i.e.* the same medium as used for blood-agar, but considerably diluted. For our continuous observations we have used hanging-drop preparations in sealed cells; in these cases we used very dilute Lemco-broth, without any agar, as the medium for the development of the flagellates, because in the denser agar-medium it is very difficult to see the flagellum. In all these media the flagellate forms the protozoan component of a "mixed culture," since there is, of course, an even greater and more rapid bacterial development.

Commencing the account of the life-cycle with the permanent cyst, this is a small spherical or slightly ovoid body about 3–3½ μ in diameter (figs. 1–3). The cyst-wall is well defined, but not very thick; it appears to consist of a single membrane, there being no differentiation into inner and outer envelope such as is found, for example, in many *Amœba* cysts. Sometimes bacteria are adherent to the cyst-wall (fig. 3). The protoplasm is

* The generic name is formed on the analogy of the Homeric epithet, ἑλκεσίπεπλος, trailing mantle or cloak. We are indebted to Prof. Minchin for suggesting this appropriate name.

fairly homogeneous, or contains fine granules There is frequently, however, a conspicuous, somewhat refringent grain, situated near the periphery (Plate 13, figs. 1 and 2), but no vacuole is present The nucleus can usually be seen as a clearer area, and at times the contained karyosome can be distinctly made out as a dull body in the centre No division takes place inside the cyst, which is therefore a resting or "Dauer" cyst and not a multiplicative one

Excystation.—We have followed the process of excystation in cysts which had been for about 22 hours in fresh medium (Liebig broth) This is not quite the minimum period required, before the flagellate will emerge from its cyst, for in this particular preparation some half-dozen individuals were already active by this time, though the great majority were still encysted. The period which must elapse depends, we consider, upon how soon the multiplication of the active bacilli in the new environment has taken place to a sufficient extent to produce, in sufficient quantity, the ferment or chemical substance in solution which is either directly or indirectly the cause of the dissolution of the cyst-wall For we are strongly of opinion that the explanation of both the encystment and the excystation of *Helkesimastix* (and probably equally of other dung- and infusion-flagellates) is to be found in the view put forward by Cropper and Drew* in their recent important and suggestive work on the causative factors of the corresponding phenomena in *Amœbæ*. We hope to study our new flagellate from this biological standpoint subsequently, and will here merely indicate in passing certain facts we have observed which bear upon the question. We have found excystation occurring only in those cultures, plates, or observation-preparations in which there was a plentiful development of an (or of more than one) active, markedly aerobic bacillus, which we have not yet more closely determined We have kept cysts in ordinary cover-slip preparations in different media (the preparations being sealed, of course, to prevent evaporation), for several days, without the emergence of any active individuals taking place, and in such preparations, the medium not being in contact with free air, no noticeable development of active bacilli took place.

In a cyst from which the flagellate is about to be liberated the wall is noticed to become gradually less and less obvious, until at length one can no longer say that there is any envelope present, apart from the delicate membrane or pellicle limiting the body-protoplasm (fig. 4) There is no definite rupture or bursting of the wall at any point, through which the flagellate could pass out to the exterior, in other words, when the creature has again become active and moved off, there is no cyst-wall left behind. We were the

* 'Researches on Induced Cell-reproduction in Amœbæ,' London, John Murray, 1914.

more convinced of this because we had previously seen the excystation of a species of *Bodo* from other dung-cultures, and in that case the rupture of the cyst-wall was very evident, and when the *Bodo* had moved away the empty cyst-wall was left behind To return to *Helkesimastix*, when a sphere from which the cyst-wall had disappeared was watched it was seen gradually to change its shape, and in about seven minutes had elongated somewhat and become ovoid (fig 5). Near one extremity a small vacuole had made its appearance, which represented the contractile vacuole, active metabolism had again begun Very slight, spasmodic movements were noticed at intervals, and then suddenly it was seen that a very short, delicate flagellum was present, apparently emerging from about the middle of the body (fig 6) This waved slowly to and fro, though we do not feel at all certain that it was causing the jerky movements The flagellum could at first be made out only when it was projecting from the side of the creature, and not when it lay over the body-protoplasm. Meanwhile the flagellate was growing in size rapidly, and five or six minutes later it appeared as in fig 7 the flagellum had lengthened considerably and was more prominent and stouter, and its point of origin was now near to the anterior end The nucleus had doubtless also passed towards the anterior end, but unfortunately it could not be clearly made out, this was owing partly to the jerky movements and partly to the fact that the protoplasm was becoming very granular. The jerky, to and fro movements of the body, producing no displacement of the creature, are very characteristic of certain phases, we describe them as "knicking" movements (*cf* below, p 358). Ten minutes later the flagellate was beginning to make short, gliding movements of progression, and these alternated for some time with the knicking movements until, after about an hour, it moved steadily for the first time out of the field of vision

During this period the same process had been going on throughout the preparation, and many actively gliding individuals were now present These were all growing rapidly before beginning to multiply, and attained a size considerably larger than the average individual size found later on, the protoplasm was usually full of refringent granules

Form and Structure—The body of the ordinary active individual of *Helkesimastix fæcicola* is typically elongated and fairly cylindrical, with one end (the anterior one) bluntly rounded, the other (posterior) one tapering away more and being at times somewhat pointed (figs 8, 10, 13) The broadest part of the body is generally nearer to the anterior end We can most aptly compare the form with that of the fleshy part of a small carrot Sometimes the hinder extremity is drawn out into a narrow prolongation (fig. 11). This hinder part of the body is often very plastic and irregular,

and reminds us somewhat of the posterior extremity of *Cercobodo* (*Cercomonas*), with its long cytoplasmic "tail," though in *Helkesimastix* it is never, in normal conditions, drawn out to anything like the same extent. The body is usually about 6μ to 7μ long by $2\frac{1}{2}\mu$ or 2μ broad. Under other conditions the shape of the body may be oval or slightly pyriform (figs. 9, 18); this is frequently the case in smaller individuals, and also when the flagellates are sluggish, in a rather denser medium than usual.

There is a single flagellum, generally about two and a half to three times as long as the body, or even longer, inserted at, or very near to, the anterior end. This new flagellate is remarkable in having its flagellum *always* directed backwards, *i.e.* it possesses only a trailing flagellum, whence the generic name. In life, the flagellum is usually contiguous to the body for practically the entire length of the latter, and the proximal portion is always in very close contact with about the anterior third or so of the body, from which it never becomes free. This is seen very clearly when a steadily progressing individual makes a sharp turn and goes off in another direction. The proximal portion of the flagellum also turns immediately, along with the body, but the remaining part is for the moment free from the latter and turns more gradually into the new direction (*cf.* fig. 14). The flagellum lies along the middle of the upper (dorsal) side of the creature; in ordinary conditions it is never on the under side. Sometimes the flagellate turns on its side, when the close adherence of the flagellum is well shown (fig. 10). Yet, in spite of this close contact, there is certainly no attaching membrane developed, for, in individuals killed by osmic acid, the flagellum is sometimes seen to stand off completely from the body (*cf.* fig. 12). Frequently there is a definite row of three or four conspicuous granules along the line of contact of the body with the flagellum (figs. 10, 11). The nucleus can generally be seen as a clear, spherical area near the anterior end; in individuals in motion it is sometimes difficult to see the karysome, but in those quiescent, or in individuals killed by osmic acid vapour, this body can also be made out (*cf.* fig. 12). *Helkesimastix* is not a binucleate; it has no kinetonucleus.*

There is certainly no cytostome or definite mouth-aperture present. We are not quite certain, however, whether the creature does or does not ingest solid particles, such as small cocci, etc.; we are strongly inclined to think it does not. In this connection, a peculiar mode of food-ingestion which we have observed in *Cercomonas* (or *Cercobodo*) *longicauda* is of interest. While

* We may add that, as seen in stained preparations, the nucleus is of the usual flagellate character, consisting of a nuclear membrane, a clear zone (the nuclear sap), and a large central karyosome, connected with the membrane by delicate radiating fibrils.

an individual was moving sluggishly along, the posterior, plastic part of the body would come into contact at some point with a small extraneous particle A small portion of the protoplasm of the flagellate in the immediate neighbourhood of the point of contact would remain adherent to the still stationary particle for a few seconds, gradually engulfing it, while the *Cercomonas* moved calmly on Thus most of the body was quickly separated from the small portion of cytoplasm remaining behind, until often only an extremely thin thread or line of protoplasmic substance still joined the two parts, this thread might be as long as the whole body of the flagellate Suddenly the tension of the connecting thread overcame the resistance of the stationary particle, and the latter, together with the small portion of protoplasm surrounding it, was safely hauled up again into the main body We often thought the thread must break, but it never did!

As our new flagellate shows a resemblance in some respects to *Cercomonas*, we have watched particularly for the occurrence of anything corresponding, which, however, we have never seen, although analogous appearances are seen during conjugation (*cf* below) We have never been able to satisfy ourselves that *Helkesimastix* does engulf solid particles at the hinder, plastic end, but do not deny the possibility of it doing so. We certainly consider, however, that its principal mode of nutrition is by osmosis The natural habitat of the creature, namely, moist dung, is, of course, rich in organic matter in solution, when the bacteria have been active for a little time In *Helkesimastix*, therefore, we have an instance of a form which is, at all events, mainly saprozoic

The contractile vacuole is usually small, that is to say, it contracts before it attains a large size. It is generally situated in the hinder part of the body, to one side (figs 8, 10). Now and again, however, owing doubtless to some condition of the medium, the contractile vacuole becomes very large (fig 15) Immediately after it has ruptured the protoplasmic wall and its contents have passed out to the exterior, the body of the flagellate presents for a short time the curious appearance of fig 16, the hinder end is forked, like the two arms of a V After a little while it becomes triangular (fig 17), and ultimately assumes again its normal shape.

Another point illustrating the looseness or plasticity of the body is a method of turning round often shown by a sluggishly moving individual It will come to rest, and the body becomes more ovoid (fig 18). Then the side with which the flagellum is in close contact begins to show what we term "working" movements, at first the peripheral protoplasm moves in slow, short, irregular waves to and fro, the line both of the contiguous part of the flagellum and of the row of granules (if these are present) becoming meanwhile indented and

uneven (fig. 19). These peripheral movements of the protoplasm can perhaps be compared, on a very small scale, with the wave-like movements of the ectoplasm of *Amœba verrucosa* Next, the anterior portion of the body-protoplasm on this side is moved as a whole backwards, around the central part, as it were, carrying with it the anterior part of the flagellum and the granules, and also, doubtless, the nucleus (*cf* fig 20). Finally, the creature elongates again, having the anterior part of its body now where the posterior part formerly was (fig 21), and is ready to swim away in just the opposite direction

Movements—*Helkesimastix* possesses two distinct and characteristic methods of locomotion One, the more usual mode, is very interesting, because it is very difficult to explain, in fact, we do not know quite how to explain it. The creature glides forwards with a steady, almost unwavering movement, the flagellum trailing behind in a straight line While the rate of progression is not as rapid as that of a *Monas* or a *Bodo*, for example, the movement cannot by any means be called slow, indeed, it is often surprisingly fast, considering how little there seems to be to account for it There is certainly no vibration of the free, distal part of the flagellum at all, in this method of movement, the flagellum does not act as a pulsellum The creature is always at the surface of the medium when moving in this way (or it is gliding along the under surface of the cover-slip), the flagellum in both cases being uppermost. This fact leads us to think that surface-tension plays some part in this type of movement. The body is, as it were, suspended along its length to the flagellar thread, as a gymnast may be suspended along a tight rope The body is often seen to swing sideways partially around its flagellum, appearing then as in fig. 10, but it never swings right above the flagellum The only movement of the body which can be noted is a very slight "knicking" movement of the anterior end, *i.e.* the anterior end may make little tentative jerks from side to side, perhaps caused by slight contractions of the anterior end of the flagellum But the anterior end of the body is not displaced laterally by more than half its width, if as much, and the strength of these slight movements appears wholly insufficient to produce the steady forward progression, moreover, the creature may glide for quite a considerable distance without even these

The other characteristic mode of progression occurs when an individual is in the middle of the fluid, *i.e* completely surrounded by it Then it performs vigorous, more or less undulatory movements of its whole body and flagellum, the latter, as regards its applied portion, never becoming separated from the body, and with its free, distal part lashing actively behind. The movement of the body is very like that of a fish's tail, and not at all unlike the

movements of certain trypanosomes. Yet, in spite of all this activity, the rate of progression is no greater than is obtained by the quiet surface gliding

One other variety of movement—not of progression—remains to be noted. An individual which has been gliding about will become anchored by the end of its flagellum to some particle of débris or small clump of bacteria. The body will then execute sharp bending or knicking movements about its narrow, posterior end, where the flagellum becomes free, often turning through nearly 180°, and then turning back again This anchoring process recalls the anchoring of some Bodos by the trailing flagellum, but the body movement in *Helkesimastix* is not at all of the vibrating or dancing character seen in the *Bodo*, because it lacks the anterior, vibratile flagellum

Multiplication—After the flagellates have been active for some hours multiplication begins We have not observed it occurring up to six hours after excystation has taken place, but by the end of 20 hours it was proceeding actively and had evidently been going on for some time. An individual about to undergo division always comes to rest in the first place The body becomes ovoid and then practically spherical (figs. 22, 24, 27) The subsequent course of events is not always quite uniform, though the variations are only slight In the great majority of cases, by the time the body-form has become rounded, or even after it has been ovoid for a short while, the flagellum is no longer visible The free part has entirely disappeared, and we are strongly inclined to think that the attached part also goes, though we cannot write with absolute certainty because it is extremely difficult to detect a motionless flagellum lying over, and closely applied to, the body However, we think that, in many cases, it is probably entirely withdrawn and absorbed, especially as, just prior to this stage, small "working" movements of the peripheral protoplasm occur at the side where the flagellum lay At this stage the clear nuclear area can frequently be seen to be elongated and to possess now two karyosomatic bodies (fig. 22), the parent-karyosome having already divided. Next, the body begins to elongate again (figs. 23, 29), and in a minute or two more becomes slightly dumb-bell shaped (fig. 25) The two daughter-nuclei are practically reconstituted and have begun to separate by this time (figs 23, 29), they can usually be seen because the body remains motionless during this period. (We hope to give information with regard to the cytological details both of multiplication and conjugation in a subsequent memoir, when we have studied fixed and stained preparations). The time of appearance of the first new (daughter) flagellum varies somewhat. As a

rule, it does not appear until the dumb-bell stage is reached, when it can suddenly be seen projecting out from the side of the body, a short distance from one end (fig 23), and waving slightly to and fro Occasionally, however, it can be seen while the creature is still rounded (fig 24), and in such a case it may represent the old flagellum, which has been only partially withdrawn The constriction at the middle of the elongated body now rapidly increases (figs 25, 30), and about this time the second flagellum appears, always some distance away from the first and in the other half of the body, not far from the second nucleus. Very generally, the second new flagellum projects out from the side of the body opposite that where the first one is. The flagella increase in length and the body undergoes little irregular, jerky movements. Its middle part becomes narrower and more drawn out, the whole body having now the appearance of a double pear. Usually the two flagella have their free ends directed towards the middle, the bluntly rounded extremities becoming the anterior ends of the two daughter-individuals, though sometimes the second daughter-flagellum (the later developed one) starts from near the middle of the body, *i.e.* near to the constriction connecting the two halves, and points outwards. Ultimately, helped by the movements of the flagella, the two halves of the body are drawn still farther apart and the small daughter-individuals at length separate, gliding away in opposite directions. The cytoplasmic "tail," which each at first possesses, rapidly contracts and the typical body-form is attained (fig. 31)

In the above type of division, which is the most usual one, we regard the cytoplasmic fission as being approximately transverse to the original long axis, so that in this case we have the flagellate undergoing transverse division of the body. The whole process is fairly rapid. From the time when an individual has become ovoid and practically motionless to the time of separation of the two daughter-individuals only 10 to 15 minutes usually elapse. Now and again, however, the process is slower, taking upwards of 25 minutes; but this is of rare occurrence. In such a case, moreover, we have noticed that the body becomes divided before the second flagellum is formed, so that one daughter-individual swims away, leaving the other motionless for a few minutes longer, until it has developed its flagellum.

On one or two occasions we have observed a modification of the above method of division, a second flagellum being formed while the old one is still present, having only been withdrawn (shortened) a little (fig. 32). The shortening proceeds further (fig. 33), but in this case the old flagellum is not entirely absorbed but forms the basis of one of the daughter-flagella. In this variety of division we regard the fission of the body as being more in the long

axis, rather than transverse This may represent a more primitive mode, which has been largely relinquished in favour of the other

Syngamy.—In a fresh culture (either agar-plate or observation-preparation), after the flagellates have once emerged from their cysts, multiplication goes on, often at first with amazing rapidity, for two days or so, until by about the third day—or sometimes even earlier—an epidemic of conjugation sets in The only important point in the whole life-cycle in regard to which we are not yet certain is whether definite conjugating individuals, gametes, morphologically distinctive from the usual forms, are developed, and, if they are, whether these are anisogamous or not Our difficulty arises from the fact that in a culture in which conjugation is beginning, the flagellates present show more or less the customary variation in size and form, and, further, we have not yet succeeded in seeing two individuals actually come together and unite. In most of the cultures in which we have observed conjugation, the majority of the individuals belong to one of two slightly different types One of these is rather characteristic and distinctive, we think, of this period It has the posterior end of the body gradually tapering and always turned definitely to one side (figs. 34, 35), usually, though not invariably, the right side, when the flagellum is dorsal or uppermost The curved tail-portion differs from the irregular extension sometimes seen at the hinder end in ordinary individuals (*cf* fig 11), in being fairly constant and not so changeable or metabolic, now retracted and now drawn out, as in that case. The other type is distinctly smaller, but is not so readily distinguishable from an ordinary individual (fig 36), it is oval in shape, and the hinder end is usually more bluntly rounded.

In an observation-preparation in which syngamy has begun, two individuals are often noticed to come into contact and glide along together for a short while (fig 37) The members of such a pair are very frequently—though, again, not invariably—of the two distinct forms just noted. The larger one of the two often appears to stick, or become attached to the other by its curved, hinder end (fig 38), when this happens, both individuals get very excited and actively jerk themselves about for a moment or two, then they will either separate abruptly or glide along together for a short distance again, and then move apart. Unfortunately, we have never seen this process followed by actual union, and therefore cannot say whether it represents a tentative seeking out of each other by definite gametes Only in one case, up to the present, have we caught the two gametes in close contact, with the body-protoplasm of each still separate just for an instant before joining up (fig 39), and, in this case, so far as could be gathered from our momentary impression before the two protoplasmic masses ran together, as it were, into

one, the two conjugating individuals were not very dissimilar. We leave the matter there for the present, but hope to settle it before publishing our detailed account.*

Of course, the actual coming together and uniting is a matter of a few seconds, and therefore difficult to catch, but the gradual fusion of the two gametes and the subsequent development of the zygote is a long process, and we have observed every stage in it, on many occasions. The actual cytoplasmic union is lateral (fig. 40), and immediately after it has occurred one would think that a single protoplasmic entity was now constituted. But the subsequent behaviour is amazing and unique, so far as we are aware, in the history of conjugating elements, and well illustrates the looseness of the first union and the fluidity of the protoplasm in *Helkesimastix*—and, we doubt not, in other of these dung- and infusion-flagellates. As soon as the actual cytoplasmic union has occurred, the definite form of the two gametes is practically lost, and there is for some time a remarkable lack of attraction, or reluctance to unite, between the two essential parts, if we may thus regard the nuclei and associated elements together with the portion of cytoplasm immediately surrounding them. (This is probably because the nuclei have not yet undergone a process of maturation.) We cannot do better than describe the sequence of form-changes undergone by the zygote immediately following the union, in the instance referred to.

The conjugants of fig. 40 had not been joined for more than a minute or so before they separated again in the anterior region, one individual being desirous of steering off to the right, while the other preferred to keep straight on (fig. 42). This little difference being composed and the two individuals joined up again, one began to lag behind the other, the combined body appearing now like an irregular rhomboid (fig. 43). The conjugants were progressing forwards, more or less steadily, all the time. In the next instant the smaller half had slipped still further back, and the body had now the appearance of fig. 44. A few seconds later it was quite behind the larger half (or individual), the two being connected together only by a narrow cytoplasmic thread (fig. 45). After progressing thus for a short distance the smaller half rapidly overtook the one in front by a kind of "slithering" movement along it, and the protoplasm of both again joined up along the side (fig. 46). A few seconds later a very characteristic stage was reached, in which the combined body of the two conjugants

* We have since reconsidered the above point and now think that the tendency to adhere in couples in this way is probably purely a matter of surface tension or attraction. We have observed the same phenomenon in observation-preparations of a non-conjugating strain of a closely allied species (*vide* p. 366, *et seq.*).

was almost square, the whole zygote, with its two flagella trailing along near the outer sides, having almost the appearance of a procession banner (fig. 47). (At a later stage the body becomes more definitely rectangular and banner-like.) In this particular instance the zygote remained thus for a couple of minutes or so, moving along steadily, the two "halves" with their flagella at times approximating slightly, causing the common cytoplasm to sag, as it were, just as a banner does when its pole bearers do not keep their line Now and again the zygote turned on its side, when it appeared as in fig 41 At length it altered again completely, a portion of the body, apparently about a third of the bulk, advancing quickly in front of the remainder (fig 48), until the two portions were only connected by a very narrow thread of cytoplasm, which was at first extended along and in contact with the greater part of the flagellum (fig 49) We have no doubt that in this remarkable dissociation of the zygote into two portions, one nucleus goes with each half, just as the origin and proximal part of the flagellum can clearly be seen to do Unfortunately, we could not make out the nuclei at all during these living observations of the conjugation-processes, this was due partly to the fact that in the earlier stages the conjugating pairs are very active and constantly undergoing form-changes, and partly because, in the later stages, the nuclei are most probably undergoing maturation prior to nuclear fusion This separation, which may amount almost to disruption, of the common cytoplasm into two portions reminds us of the behaviour of the protoplasm of *Cercomonas* (*Cercobodo*) above alluded to, but in *Helkesimastix* it is not a passive leaving behind of a certain amount of protoplasm, but a separation actively brought about by a difference in behaviour of the two gametic energids. Here, again, in more than one instance (*cf* fig 54), we felt sure that actual rupture was going to occur, but we have observed this remarkable process on several occasions and the two halves never broke loose To return to our particular zygote, the small leading portion suddenly turned right round, its flagellum becoming at the same time mostly free from the cytoplasmic thread (fig 50), and then joined up again to the main portion (figs. 51 and 52) The zygote next assumed the form of a pear (fig. 53), and after another minute or less the stage of fig. 45 was repeated, with the difference that the smaller half was this time in front. The hinder part then "slithered" up along the other and the banner form was again arrived at

All the above changes took place in a period of about 14 minutes from the instant of first union. This time the banner was fairly permanent, and after following it for a little while longer we left it, as we knew exactly what the subsequent behaviour would be from numerous earlier observations. The above described remarkable behaviour of the two conjugants—or, at least, of

the two essential portions, since the cytoplasm is apparently indiscriminately divided at times—is not at all an exceptional occurrence; indeed, we think something similar usually happens during the earlier stages of syngamy. On other occasions we have seen both the "slithering" of two slightly unequal portions and the almost complete separation of the two halves which remained connected only by a delicate thread, just as in the above case The joining up again in such a case occurs, we consider, as a result of the rapid contraction of the connecting thread, in just the same manner as the lagging portion of protoplasm was suddenly hauled up into the main body of the *Cercobodo* (*cf.* above) Further examples of these early conjugation stages are given in figs. 54–57, from different zygotes Even after the banner has been definitely formed for as long as an hour, we have seen it suddenly break down into two portions, one behind the other.

Different forms assumed by the banner, when at length permanent, are seen in figs 58–62 As time goes on the banner gradually loses its square shape and becomes oblong (fig. 63), the two parallel flagella also gradually coming to lie nearer to the mid-dorsal line, and so to each other. Most probably, by this time, the maturation processes of the gamete-nuclei are completed Further, the two flagella are slowly shortening in length. In its earlier period, however, the banner is still capable of performing the active, undulating (free) movement, as well as the more characteristic gliding movement. From an oblong the banner now turns to an oval—really, of course, to an ovoid—which is ultimately almost as deep as it is broad We have followed a banner from the time when it had about just become permanent (*e.g* as in figs. 61 and 62), up to the definite oval (fig. 65), the time occupied being about three hours. In an observation-preparation in which syngamy has been going on for some time, banners and ovals are quite numerous; this will be apparent when it is remembered that every cyst formed is a zygote-cyst At a later stage the two flagella have become much shorter (figs. 66 and 67), and the flagellate moves very sluggishly; it soon ceases to displace itself and only performs little turning and "knicking" movements. The protoplasm becomes contracted and somewhat denser, and the oval nearly always leaves the under surface of the cover-slip by this time and sinks to the lower level of the medium. The flagella are at length quite absorbed and the body becomes spherical. We have followed an individual oval with two flagella close together, as in fig. 65, up to the time when it became a motionless, rounded body at the lower level, and this change took about six hours. The whole process up to this stage takes from 9 to 10 hours, according to our actual observations; of course, this may not be the minimum period required, though we should say it is not far from it.

We have not actually watched a motionless, rounded zygote through its encystment period, but we noted the position of the above individual and of others at the same time late in the evening, and on looking at them next morning we found they had become the very characteristic "shrinkage" cysts (figs 68 and 69) The cyst-wall has been formed and the protoplasm has continued to contract, so that a space is left at one side between the body and the wall. We are inclined to think it is a space containing no liquid, because it is always very clear The space appears of different size, according to the age of the cyst (figs 68 and 69) When small the space is spherical, but when it attains its maximum size it is lens-shaped A curious fact is that after the cyst has shown this condition of shrinkage for some time—the period may vary from two days or less up to longer—the space disappears entirely and the cyst becomes a permanent cyst, as described at the commencement of this account This finishes the life-cycle

Biological Notes—As, in a normal culture, the great majority of the flagellates form cysts, one could not have a more convincing and readily obtained demonstration of the regular occurrence of syngamy in the life-cycle of these simple dung-flagellates Although all the above observations on *Helkesimastix* have been described either from plate-cultures or from observation-preparations, this is only because, on account of the more rapid multiplication and development under those conditions, the individuals are very much more abundant than in a simple, moistened dung-culture, and therefore the different stages are more readily found We have not the slightest doubt that the life-cycle is exactly similar under the more natural conditions, the only difference being that it is probably slower, *i e* a longer period may elapse before the whole life-cycle is completed. For instance, it is usually three or four days before this flagellate is observed in the active condition in a dilute dung-culture, and it may persist in the active condition for a week or more before forming cysts. The reason for this is, we consider, because the watery medium is not nearly so rich in nourishment as the beef-extract medium. The bacterial development is not nearly so great as in the latter case, nor is the multiplication of the flagellates so abundant Therefore, on the one hand, a somewhat longer time most probably elapses before the cyst-wall is dissolved, and, on the other hand, the medium does not so soon become excessively full of the "toxic" products, or whatever chemical substances induce the cessation of multiplication and the tendency to conjugation, whether formed from the flagellates, or the bacteria, or from both As, however, we are at present engaged in making a full study of this interesting flagellate from a biological standpoint we will not further discuss these questions at present.

Loss of Syngamy and the Power to form Cysts.—We have discovered one very interesting and important fact, however, which deserves to be mentioned. By sub-culturing the flagellates* while they are still all in the active condition, *i.e.* before cysts have been developed, on to a fresh plate of the same medium, we have found that multiplication will continue for a further period (of two days or so), before conjugation sets in. The important fact, however, is that, after a certain number of sub-cultures have been thus made, the flagellates, although they are still able to thrive and multiply actively on each successive transference, no longer undergo syngamy, followed by cyst-formation, and have, so far as can be seen, entirely lost the power to do so, at all events under the existing conditions.† Up to the present time (October), we have thus kept a "strain" for more than 20 weeks, through 35 sub-cultures on to fresh "constant" medium, and in each sub-culture the flagellates have multiplied enormously. In the sub-cultures up to the fourth a very few isolated cysts were still found. *But in none of the subsequent ones has a single cyst ever been seen.* The flagellates, instead of conjugating and forming great numbers of cysts, as usual, degenerate and die off, only a small proportion remaining still alive. Yet, at the end of 10 or 12 days, sometimes more, a few individuals still persist alive, and the transference to a fresh sub-culture can be successfully made. The flagellates which remain alive are rounded, granular, very sluggish forms, with the flagellum sticking straight out and almost motionless; but, in the fresh medium, they are capable of quick recuperation and renewed multiplication.

During the earlier weeks, after this new strain was fairly started, we never observed any biflagellate forms, comparable to the conjugating pairs above described, although thousands of individuals must have passed under our eyes. Then, during the 8th and 9th weeks, respectively, we observed on two occasions, in different observation preparations, a single biflagellate individual. After this, we looked carefully for others, at intervals, but no more were seen until the 14th week (in the 23rd sub-culture), when another was observed. Since that time, in most of the sub-cultures, a few biflagellate individuals (banner-like forms) have been found and the number of these has gradually tended to become less infrequent, although they still constitute a very small

* This experimental work, it may be mentioned, has been carried out upon another species of *Helkesimastix*, with which we have worked latterly. This form (*H. major*, n. sp.) also occurs both in sheep and goats, and differs only from *H. faecicola* in the larger size of the adult individuals and also of the cysts, rendering it more convenient for study. The life-cycle is similar in both.

† We tried the experiment of re-introducing the non-conjugating "strain" into the original dung culture (sterilised) with the object of seeing whether the ability to undergo syngamy would be restored. This also gave negative results.

proportion only of the total number of flagellates present We have been able, on several occasions recently, to follow the further behaviour of these forms, and have ascertained the important fact that they always divide, and never become rounded-off and form cysts. Moreover, we have observed a certain number of these forms which were very large and possessed not two, but three, or even four or five flagella (and nuclei, as ascertained from permanent preparations) We have seen an individual with three flagella, and also one with five, actually divide into two rather unequal portions, in the former case, one half had two flagella and the other one, and in the latter case, one had three and the other two flagella (and nuclei).

We will assume, for the present, that these forms represent a union of two or more individuals, rather than a long-delayed division * When we first observed the isolated instances of biflagellate forms in this strain, we considered that they represented true syngamy, which, although occurring very rarely was apparently still "latent" We now know (1) that these "unions" may be either binary or multiple, apparently more or less indifferently, (2) that these forms always divide, the nuclei and flagella being apportioned out, often unequally, between the two halves, and that they never proceed to form cysts—the invariable sequel, normally, to conjugation in *Helkesimastix*, (3) and, lastly, that these forms, or the products of their division, are equally incapable of persisting ultimately (unless, of course, they are transferred to fresh medium) Hence, we feel certain that none of these unions represent true syngamy Bearing in mind the extremely plastic character of the cytoplasm of this creature, we consider these unions are due to physical, rather than "vital" factors, and result from continued cultivation and the condition of the culture at the time

In our opinion, these peculiar cases do not invalidate the following general conclusion which we wish to draw from our observations on this non-conjugating strain. The "intensive" culture of this flagellate has resulted in the loss of the power to undergo true syngamy and to form cysts The further existence of this "strain" is now dependent on continued transference to fresh "constant" medium Minchin, in his 'Introduction to the Protozoa' (London, Arnold, 1912), in the chapter on "Syngamy and Sex," has pointed out (p 161) that "intensive culture, whether artificial or

* We cannot yet write with certainty upon this point, because we think, from the evidence obtained so far, it is possible that these forms represent a long-drawn-out mode of division, due to the effect of the cultural conditions, in which the nucleus and flagellum divide, it may be more than once, before the cytoplasm does. There are other cases known of a corresponding behaviour under conditions which are probably not quite normal; an example which is particularly interesting in relation to the present discussion is that of multiple longitudinal fission in certain lethal trypanosomes

natural, as in parasitism, seems to diminish the necessity for syngamy"; from this stage it is only a step further to find the capacity for syngamy lost.

Bearing on the Case of the Trypanosomes.—It appears to us that this remarkable experimental fact has an important bearing on the question of the trypanosomes, and may afford an explanation of why conjugation (or syngamy) in these parasites, though assiduously sought, has not been observed in any authenticated instance. Probably it never does occur, because these forms have lost the power to conjugate. Just as, in the case of the above-discussed strain of *Helkesimastix*, the rapid, successive transferences to fresh, non-toxic medium at first removed the necessity for conjugation (and encystment), and then have led to the loss of the ability to undergo syngamy (and, in this case, to form cysts), so a similar development has very likely occurred in the trypanosomes and related forms. As is obvious, the conditions of life in these parasites are readily comparable to those above described. The trypanosomes live in a rich nutritive medium, namely blood, in the vertebrate, or blood in various stages of digestion, in the invertebrate host, and, as is well known, rapid multiplication in both hosts is usually found. In the invertebrate we get, more or less frequently, replenishment, *i.e.* a fresh supply of the "constant" medium (namely blood), together with removal of toxic products. In the blood of the vertebrate, where there is at first abundance of medium, but not any "fresh" supply, it is very interesting to note that, in the case at all events of many lethal trypanosomes, which, as Minchin has pointed out, are not yet completely adapted to their hosts, we get the production eventually—after active multiplication has gone on for some time—of the well known "involution" forms. These, at any rate in our opinion, as in that of many French workers, simply die off after a time, if left to themselves, but they are capable of being "rejuvenated" and of again multiplying actively if passed into a fresh host. And a similar state of affairs has been met with by one of us in cultures of avian trypanosomes. Now it seems to us that these two cases present a very close parallel to what we have observed in *Helkesimastix*. It may be pointed out, also, that the non-conjugating strain of *Helkesimastix* is the result of an abruptly originating and more or less artificial intensive culture, whereas in the case of trypanosomes and allied parasitic flagellates the corresponding conditions have operated naturally and over a long duration of time.

On the supposition that syngamy has now been entirely lost in the hæmoflagellates, the explanation which we here put forward is much more probable, we think, than the idea, which has also been suggested, that the stimulus afforded by the change of hosts is accountable for the loss of this process in the trypanosomes. We have always considered that there are two or

three serious objections to this latter view. Briefly stated, these are as follows:—(1) Trypanosomes can be successfully inoculated, without limit, into fresh vertebrate hosts, *i.e.* the same "constant" physiological medium, without showing (so far as is known) conjugation. (2) Binucleate flagellates of insects alone (*e.g.* certain leptomonads) have no alternation of hosts, and syngamy is apparently just as little likely to be found in these parasites as in the trypanosomes themselves. (We are inclined to think, indeed, that syngamy may have been lost in the ancestral trypanosome form, before ever it acquired an alternation of hosts.) Lastly, we have the important case of the Hæmosporidia, intracellular parasites which all have the change of hosts, and in all of which conjugation regularly occurs. Why should not the physiological stimulus afforded by the change of environment have influenced them also? And, on the other hand, there is no reason to doubt that the primitive ancestors of the trypanosomes underwent a process of syngamy, since it appears likely that many of these lowly protomonadine forms, among which the origin of the trypanosomes is to be sought, possess this feature.

We consider, therefore, developing the ideas expressed by Cropper and Drew (*loc. cit.*) along the line indicated by the experimental facts adduced above, that the loss of syngamy is due to the surfeit of nutrition, together with the non-toxicity of the medium, that is to say, the absence (in excess) of the chemical substance or substances to which the flagellates react *normally* by the cessation of multiplication and the onset of conjugation. It will be readily apparent, from what has been just pointed out, how these factors have prevailed in the case of the trypanosomes. And we think that a similar explanation can be applied, not only to the case of insectan Binucleata, but probably to that of many other parasitic flagellates as well.

EXPLANATION OF FIGURES.

All the drawings, with the exception of fig 12, are from sketches made directly at the time of the living observation. Fig 12 is a camera lucida drawing of an individual fixed with osmic acid vapour The magnification throughout is approximately 2250 times linear, arrived at after a comparison of other individuals fixed with osmic. We are indebted to Miss Rhodes for kindly tinting and sharpening up the drawings.

In our figures, we have shown the nucleus or omitted it, according as to whether we were able to observe its position in the particular individual represented, or not. As mentioned in the text, we were unable to make out satisfactorily the nuclei in life, in the conjugating pairs

Note with regard to the flagellum —As mentioned in the text, the length of the flagellum varies in individuals otherwise similar In order to save space, we have drawn the flagellum usually short, except in fig 10, the flagellum of the individual of fig. 12 is, of course, natural length.

Plate 13.

Figs. 1–3.—Cysts.

Figs. 4–7 —Excystation and the development of the flagellum

Figs. 8–11.—Different forms of individuals.

Fig 12 —Individual fixed with osmic acid vapour, to show that the flagellum is not actually attached by any membrane to the body

Figs. 13 and 14.—To illustrate the behaviour of the flagellum in turning of the body.

Figs. 15–17.—Rupture of a large contractile vacuole and form-changes of the body.

Figs. 18–21 —To illustrate a mode of turning of a stationary individual (see text).

Figs. 22–26, 27–31, and 32 and 33.—Different instances of division (binary fission).

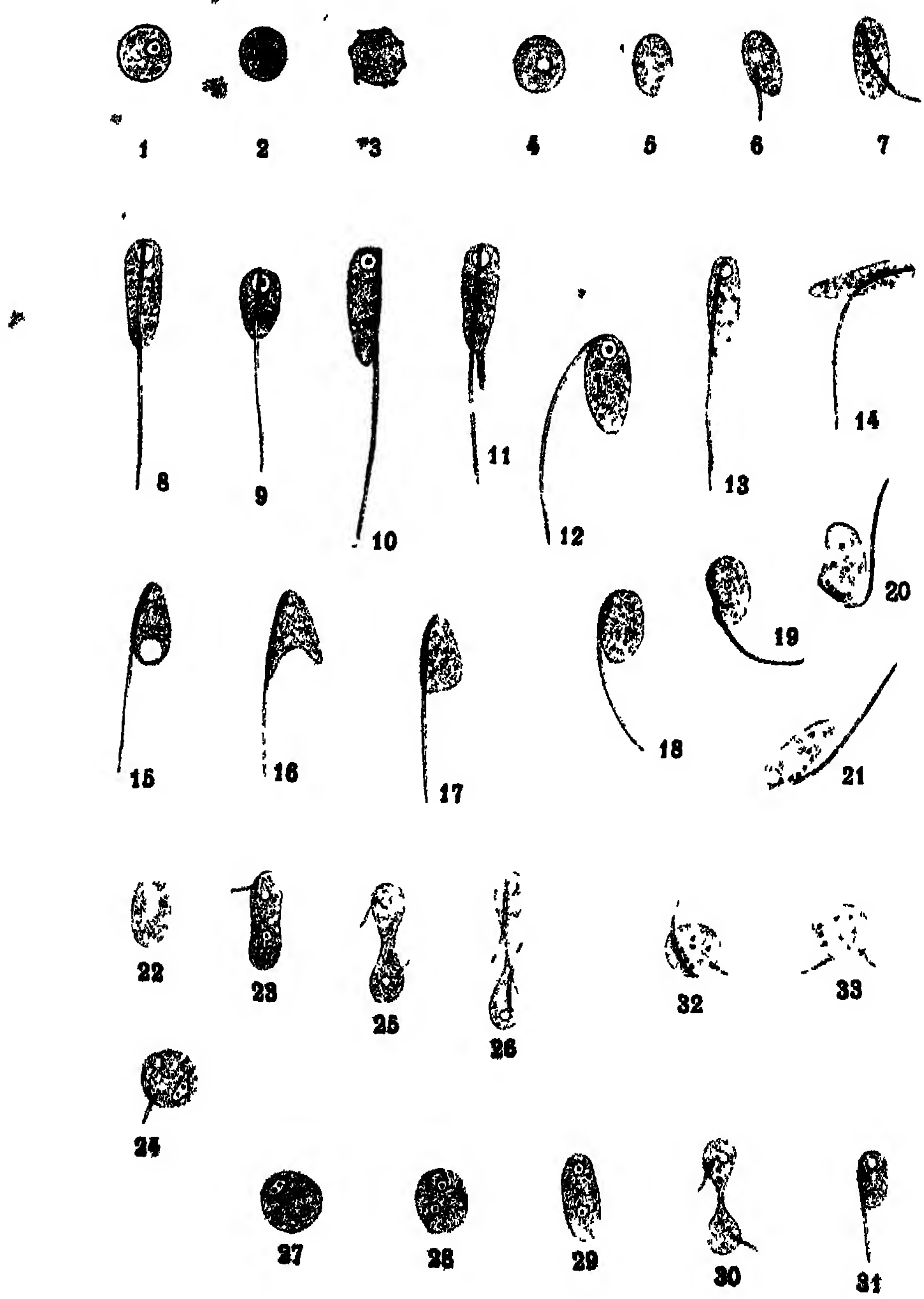
Plate 14

Figs. 34–36.—Different forms predominating in a culture about to start conjugation.

Figs 37–62.—The whole process of conjugation (syngamy), showing the different form-changes during the progress of the fusion.

Figs. 63–67.—The gradual contraction of the body and absorption of the two flagella of the zygote, prior to encystment.

Figs 68 and 69.—The cysts as first formed, so-called "shrinkage" cysts, with a vacuole or space of varying size.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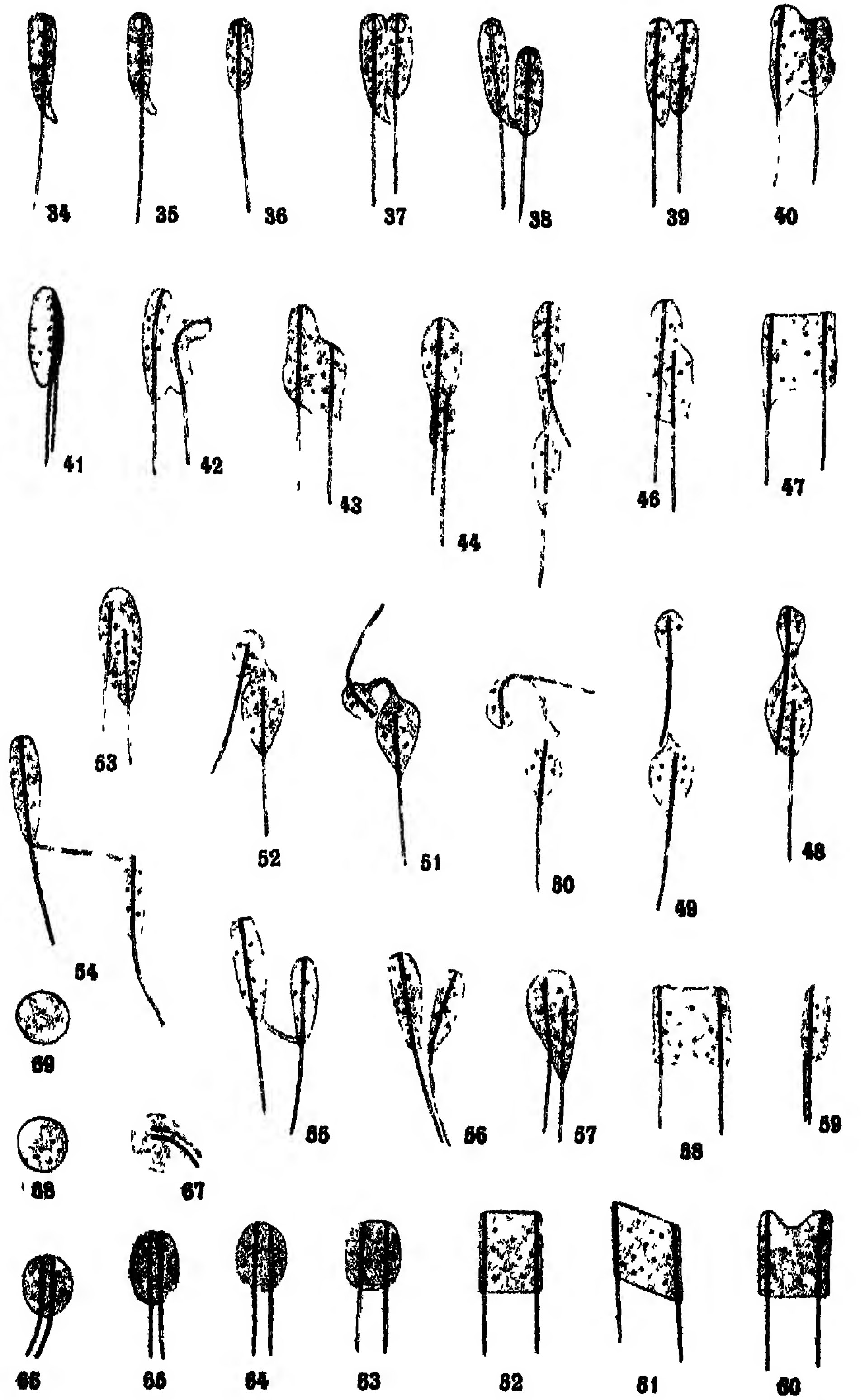
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The Antagonistic Action of Carbon Dioxide and Adrenalin on the Heart.

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(Communicated by Prof Starling, F.R.S Received August 14, 1914)

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Although a great volume of work on asphyxia has been published, it is only comparatively recently that attempts have been made to dissociate the influence of various factors in the production of the phenomena observed Kaya and Starling (1) were the first to differentiate the effects of lack of oxygen and excess of carbon dioxide in the spinal animal, and their work was elaborated by Mathison (2, 3), in whose papers a full discussion of the previous literature will be found. He found that during nitrogen administration no increased output of the heart is seen in the early stages of asphyxia, and attributed the increase in output noticed in ordinary asphyxia to the presence of increased tension of CO_2 in the blood, which Jerusalem and Starling (4) had shown to increase the systolic output of the cat's heart. He also observed an acceleration of the heart beat about the time of the primary blood pressure rise in asphyxia, which occurred even after removal of the upper part of the spinal cord. Since the work of v Anrep (5) and Itami (6), a third factor, variations in the secretory activity of the suprarenal glands, must be taken into consideration, and the present paper contains an account of an investigation of the action of carbon dioxide and adrenalin on the heart isolated from the nervous system.

Methods

The experiments reported in this paper were carried out mainly on dogs, a few on cats, the animals being anaesthetised by inhalation of chloroform and ether mixture, in the case of dogs after a preliminary hypodermic injection of morphine

The isolated heart-lung preparation was made as described by Knowlton and Starling (7). The systemic blood was taken off by a cannula in the brachiocephalic artery after ligation of the left subclavian artery and the aorta beyond; and was returned to the heart by a cannula tied in the superior vena cava after ligation of the azygos vein. The mean blood pressure was recorded by a mercury manometer connected to the side of the innominate cannula, and the side pressure of the blood from the venous return by a water manometer connected with a cannula tied in the inferior vena cava close to its entrance into the right auricle To the top of the water

manometer a small piston recorder was attached for graphic records, 0·1 grm. of hirudin was added to the blood to prevent clotting.

In the case of cats, the systemic schema (consisting of the arterial resistance and venous reservoir) used was that described by Knowlton and Starling, while the other experiments were made at various times with the different modifications of method which have marked the evolution of the systemic schema, and which have been reported in the communications from the University College laboratory during the last two years. References to these will be made in the discussion of results, as the separate points brought out are considered

In all cases the systemic output was measured directly into a graduated vessel by means of a stop-watch, and is recorded in cubic centimetres per 10 seconds, the output per minute being this observed figure multiplied by 6

When the heart volume was recorded, a glass plethysmograph on the ventricles was used similar to that described by Henderson (13), and the cardiometer was connected by air conduction with various recorders.

In some experiments the mean pressures in the left auricle were recorded from a water manometer connected with a small cannula tied in the appendage of the left auricle.

The adrenalin (Parke, Davis and Co) was mixed with the blood in the venous reservoir, while the carbon dioxide was administered from a gas bag. The figures given of the percentages of CO_2 in the gas bag can only approximately show the percentage in the air at the trachea, as various types of artificial respiration apparatus were used, and were connected to the trachea by sometimes considerable lengths of piping, and as the side slot of the tracheal cannula was more or less open.

Results.

In the heart-lung preparation, as in the intact animal, there are three circles of blood from one side of the heart to the other, one from the right ventricle to the left auricle through the lungs and two from the left ventricle to the right side of the heart, one of which passes through the schema and represents the systemic circulation in the whole animal, and the other passes through the coronary circulation. The total output of the left ventricle consists, therefore, of the systemic output, which was measured directly in our work, together with the output through the coronary vessels, so the question of the relation of the coronary output to the systemic output under normal conditions and under the influence of carbon dioxide and adrenalin must first be considered.

Relation of Coronary Sinus Flow (as Index of Total Coronary Output) to

Systemic Output.—Evans and Starling (8) found a constant relation between the flow from the coronary sinus and the total coronary output in the proportion of 3 : 5, Markwalder and Starling (9) proved that the coronary flow depended on the arterial pressure, and, using the above figure as a basis for calculation, showed that the total output from the left ventricle was constant for a given venous inflow and independent of the arterial resistance within very wide limits They have confirmed also the observations (10) made previously, that adrenalin causes an increased coronary flow

Table I* contains the results of an experiment carried out on the heart-lung preparation in which a Morawitz cannula was introduced into the coronary sinus, and the blood flow through the coronary sinus and that through the systemic part of the schema were measured at the same time The figures in column 10 (Total coronary circulation) were obtained by multiplying the observed coronary sinus flow by the factor 5/3 It will be seen that in the two series with the normal heart, the coronary output depends on the aortic pressure, while the total output is independent of the arterial resistance within wide limits and is conditioned only by the venous inflow During the period of administration of carbon dioxide the coronary flow was the same as in the normal condition but it was increased markedly during the recovery period from carbon dioxide, while the heart was returning to its normal state This increase was probably due to the accumulation of 'metabolites' in the heart during the action of CO_2.

Adding adrenalin to the circulating blood caused a great rise in the coronary flow, and this occurred also when adrenalin and carbon dioxide were given together.

We have thus a guide to the interpretation of the results obtained in other experiments where the systemic output only was measured

Systemic or Effective Output with Carbon Dioxide and Adrenalin Experiments on Dogs.—The figures in column 7 of Table I show that the administration of CO_2 may cause a marked falling-off in the output per minute This diminution may be small with low percentages of CO_2, but I have never observed an increased output during the administration The diminution becomes more marked as the percentage of CO_2 is increased, so that if the CO_2 is strong, or a moderate percentage is administered for a

* In this and the other Tables, the following abbreviations are employed.—A R. = pressure in mm Hg in air chamber surrounding the thin rubber tube forming the arterial resistance B.P = mean arterial pressure in mm Hg as measured in the cannula in the innominate artery I.V C. = pressure in inferior vena cava in mm H_2O V S = venous supply =, +, − = maintained constant, increased or diminished. Systemic output = output in c.c per 10 secs. as measured on the venous side of the artificial peripheral resistance.

Table I.—Dog, 9 8 kgrm. Heart, 142 5 grm Heart-lung preparation, with Morawitz cannula in coronary sinus.

Temp	A.R.	B.P	LVC	V.S	Rate 10 secs	Output: Systemic in 10 secs	Output: Per beat	Output: Coronary sinus 10 secs	Output: Total coronary 10 secs.	Output: Total output 10 secs.	
						c.c	c.c	c c	c c	c.c	
36° C	40	68	18		28 (alt.)	72 5	3 15	3 2	5 ·3	77 ·8	
	80	112	20	=	12 (2 1)	64	5 3	5 ·25	8 7	74 ·7	
	120	150	24	=	12 (2 1)	67	5 5	7 ·6	12 7	79 ·7	
	162	192	24	=	28 (alt.)	59	2 56	11 ·6	19 ·3	78 8	
	40	74	10	=	24	68	2 ·84	3 ·9	6 5	74 5	
	40	88	24	+	25	204	8 15	5 ·25	8 7	212 ·7	
	80	112	24	=	25	216	8 ·65	7 ·25	12 1	228	
	120	156	34	=	25 (alt)	184	7 35	14 7	24 ·5	208 5	
	160	192	200–100	=	25	103	4 ·1	20	33 4	136 4	
	40	90	26	=	25	210	8 4	6 6	11 ·0	221	
	40	86	60	=	20	193	9 ·65	6 ·7	11 1	204	8 per cent. CO_2.
	80	110	210	=	21	85	4 ·05	7 25	13 1	97	
	80	120	50	=	22	—	—	14 ·7	24 5	—	Recovery
	80	118	28	=	23	203	8 7	12 ·2	20 4	223 4	
	80	116	28	=	22	198	9 ·0	8 5	14 ·2	212	8 mins later.
	Adrenalin 0 1 mgrm.										
	80	114	0	=	36	182	5 ·05	15 ·9	26 5	208 5	
	120	166	10	=	35	178	5 1	27 8	54	232	
	164	210	10	=	35	167	4 ·76	42 5	70 ·7	230 ·7	
	Adrenalin 0 1 mgrm										
34°	40	92	4	=	35	200	5 7	17 8	29 8	229 ·8	
	40	90	2	=	30	196	6 ·5	20	33 4	229 4	
	80	112	6	=	30	183	6 ·1	27 8	54	237	
	120	158	10	=	29	172	5 ·9	38 5	64	236	8 per cent. CO_2.
	160	200	25	=	27	108	4 ·0	47 5	79	187	
	160	196	40	=	27	—	—	—	—	—	
	40	78	10	=	32	184	5 ·75	20 ·8	34 ·7	218 7	Recovery
	40	74	56	=	15 (irreg)	187	12 5	9 2	15 1	202	25 mins. later.
	80	120	40	=	20	182	9 1	13 3	22 2	204	
	120	160	50	=	20	159	7 ·96	24 ·6	41	200	

considerable time, the output of the heart may almost cease. With recovery from the effects of CO_2 the output per minute is above normal, but this occurs only after the CO_2 is removed and ordinary air respired Since the coronary output has been found not to be increased during administration of CO_2, the total output of the left ventricle is proportional to the observed output and is thus never increased but suffers more or less diminution.

After adding adrenalin to the blood circulating through a good heart, the systemic output is observed to be about the value obtained before, unless the heart was failing, but since the coronary flow is markedly raised, the total output of the left ventricle is usually increased Adrenalin sometimes can improve the condition of a heart that is working badly, or make the heart better able to work against a greater resistance, but with some hearts adrenalin is incapable of bringing about an improvement.

When adrenalin and CO_2 are combined in suitable proportions, the systemic output has been found to be increased, and, since there is also increased coronary flow, the total output of the heart may be greatly increased

Examples of such findings are given in the protocols of Table II

In Experiment 1, 5 per cent CO_2 reduced the systemic output from 130 to 96 c.c. in 10 seconds, 0·1 mgrm adrenalin also reduced the output to 103 c.c. per 10 seconds, while with combination of the two together the systemic output was 125 c.c. In Experiment 5, combining CO_2 and adrenalin increased the systemic output to more than normal Experiment 2 shows the greater effect of larger percentages of CO_2 in reducing the output, while Experiment 3 shows that the same percentage of CO_2 has a more marked effect when the arterial resistance is high and the load on the heart consequently greater.

The constancy of the output with constant venous inflow, even in the presence of varying rate and arterial resistance, has been insisted on in previous papers from this laboratory How then is it possible for the output to be increased or diminished by CO_2, or adrenalin, with constant venous inflow? The clue to the interpretation of the results is given in the effect of CO_2 and adrenalin on the venous pressures (see below) The question is one of the diastolic pressure and diastolic filling of the heart The higher the diastolic pressure, the smaller is the inflow under a given head of pressure, and it will be seen that CO_2 raises the venous pressure, while adrenalin usually lowers it

Pressures in the Inferior Vena Cava and Left Auricle.—These pressures represent (i) the side pressure of the blood flowing into the right and left ventricles, (ii) the damming back (Stauung) of the blood in the auricles during the ventricular systole These two factors are present in the normally acting heart, but there may be also (iii) a more marked abnormal

Table II.—Systemic Output.

B P	I V C	V S	Rate 10 secs	Output 10 secs	Output beat Calculated	Output beat Observed	
				Experiment 1 (10 4 13)			
				c c	c c	c c	
132	60	=	28	130	4 65		
128	150	=	21	96	4 5	—	5 per cent CO_2
140	25	=	30	113 6	3 8		
1 c c adrenalin							
134	18	=	45	103	2 3		
136	30	=	44	116	2·6		
140	30	=	35	125	3 5	—	6 per cent CO_2.
				Experiment 2 (30 4 13)			
88	30		27	59 5	2 2		
90	35		25	56 8	2 27		1 per cent CO_2.
92	32		25	56 8	2 27	—	5 2 per cent CO_2
94	30		29	59 5	2·05	—	Normal
94	40		25	53 1	2 12	—	7 5 per cent CO_2
62	90		17	7 7	0 46	—	16 per cent CO_2
0 5 c c adrenalin 1/10,000							
90	35		27	51 3	1 8	—	16 per cent CO_2
90	35		33	51 3	1 5	—	16 per cent CO_2
90	36		43	48·3	1 1	—	Recovery
				Experiment 3 (16 4 13).—Heart 87 grm			
140	10		17	31 3	1 8		
112	120		12	0	0	—	9 per cent CO_2.
140	20		13	38 5	3 0	—	Recovery
96	12		17	37	2 1		
92	15		12	17 2	1·4	—	9 per cent CO_2
60	10		16	38 5	2 4		
50	10		12	25·0	2·0	—	9 per cent CO_2.
156	10		17	51 7	3·0		
144	100		15	23	1 5	—	5 per cent CO_2.
108	10		18	55·5	3 1		
92	150		13	14 3	1 1	—	5 per cent CO_2
46	10		17	53 6	3 1		
44	14		13	51·7	4·0	—	5 per cent CO_2
132	15		18	53 6	3·0		
1 c c adrenalin							
132	50		28	55 5	2·0		
96	55		28	60	2 2		
50	35		27	51 7	1·9		
150	55		26	51 7	2·0		
192	50		25	51 7	2·05		
124	35		24	27 3	1 05		
108	25		19	5·5	0 3	—	CO_2.
1 c c adrenalin							
140	45		18	47	2 6		
128	45		30	36·6	1 2	—	CO_2.

Table II—*continued*

B P	I V C	V S	Rate 10 secs	Output 10 secs	Output beat Calculated	Output beat Observed	
Experiment 4 (10 2 14)—Dog 4 65 kgrm, heart 52 grm, cardiometer on ventricles. 36° C							
				c c	c	c c	
88	60		26·5	109	4·1	4·0	
86	80		25	103	4·1	4·0	
86	120		24	100	4·2	4·5	8 per cent CO_2
86	60		26	111	4·25	5·5	
86	60		26	108·5	4·15	4·0	
0·5 c c adrenalin							
86	90		32·5	102	3·15	4·0	
94	140	+	34	223	6·5	6·5	
104	150	+	34	333	9·8	8·75	
106	180	full	34	333	9·8	8·75	
0·5 c.c adrenalin							
106	60	=	33	333	10·1	8·5	
102	230	=	26·5	313	11·8	10·0	8 per cent CO
(1) 108	180	=	32	333	10·4	11·5	
(2) 106	190		35	313	8·9	6·5	
Experiment 5 (29 1 14)—Dog 6 45 kgrm, heart 44 grm, cardiometer on ventricles 36·3° C							
106	190		25	190	7·6	7·0	
0·5 c c. adrenalin							
110	40	=	35	—	—	5·0	
106	80		35·5	189	5·3	6·0	
108	40	=	34·5	200	5·8	7·25	8 per cent CO_2
104	40		38	—	—	6·0	

damming back through the failure of the ventricle to pass on the blood which it receives in diastole

CO_2 causes a rise of venous pressure, which is proportional to the increase of percentage of CO_2, and which is an expression of the slower rate of inflow into the heart, and of the greater damming back due to the slowed relaxation With large percentages of CO_2, the third factor also comes in as an expression of the heart failing to pass on the venous blood it receives

The addition of adrenalin to a heart in good condition lowers the venous pressures (fig 1) Both the contractile process and relaxation are more rapid, the heart is relatively longer in a relaxed condition and offers less resistance to the inflowing blood, the heart also passes on the blood better, and the side pressure of the faster venous inflow falls. In good hearts adrenalin is able to diminish, or even counteract, the effect of the CO_2 when the two are employed together

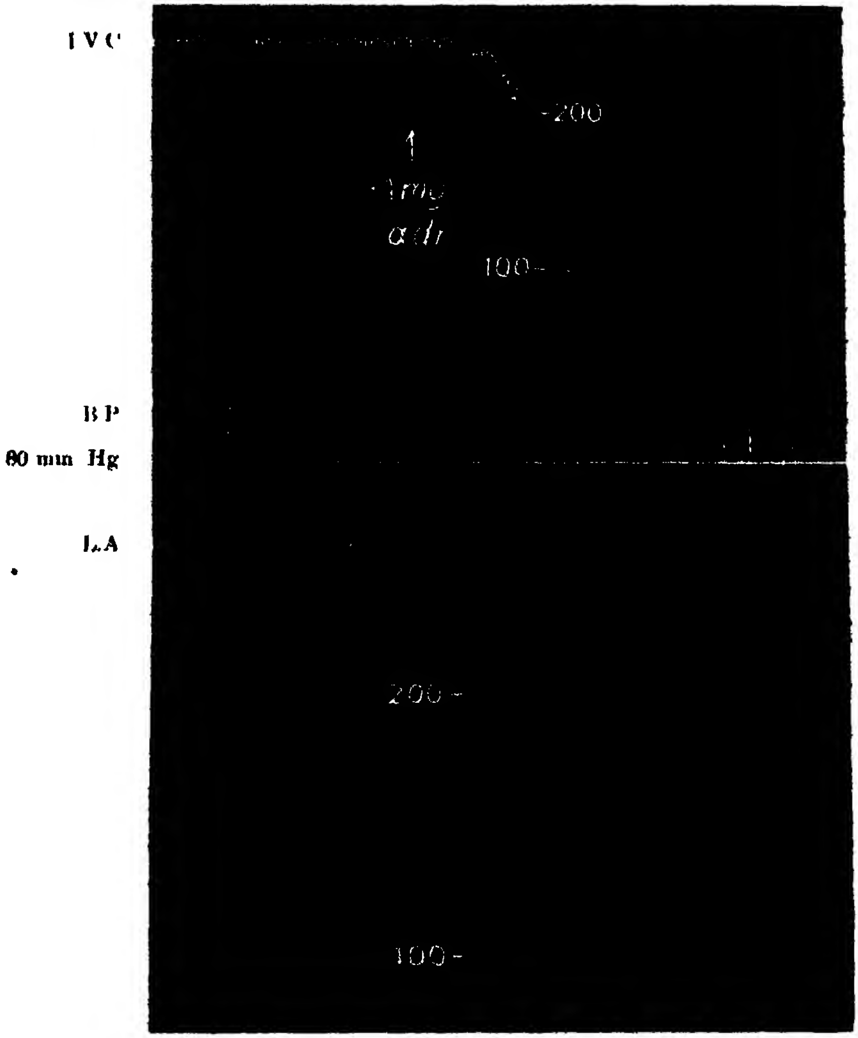

Fig 1.—Effect of Adrenalin on Inferior Vena Cava and Left Auricle Pressures. I V.C. pressure reduced from 260 mm. H_2O to 80 mm., and L.A. from 250 to 100, rate of heart increased from 22 beats in 10 secs. to 40 in 10 secs.

Adrenalin can improve a heart that is not doing well, so that it is then able to work better In Table I, adding adrenalin enabled the heart to keep up its output against a higher arterial resistance than before But if the heart muscle is doing its work badly, or if the "contractile substance" is used up, so that the heart has lost the material with which it can respond, adding adrenalin may have no good effect, and the venous pressures remain high (see Experiment 5 in Table III).

Table III.—Venous Pressures.

Temp	B P.	I V C	L A.	Rate 10 secs	Output 10 secs	Output beat
Experiment 1 (23.10.13) —Dog 6·1 kgrm , heart weight 55 grm						
					c.c	c c
35° C	126	260	500	23	189	8 ·6
	1 c c adrenalin, 1/10000					
	162	18	45	34	233	6 8
	182	14	11	37	207	5 6
	130	10	18	37	192	5 2
	180	20	40	34	189	5 5 5 per cent CO_2
	1 c c adrenalin + 8 per cent. CO_2.					
	185	16	30	38	200	5 ·2
Experiment 2.—Dog, 7 2 kgrm , heart weight 60 grm						
35 5° C	92	200	54	32	313	9 75
	1 c.c. adrenalin, 1/10000					
	92	200	14	41	263	6 ·4
Experiment 3.—Dog, 6 45 kgrm., heart weight 44 grm						
36 3° C	106	190	—	25	172	6 9
	0 5 c c. adrenalin, 1/10000					
	106	30	—	35 5	190	5 3
	108	40	—	34 5	200	5 8 8 per cent CO_2.
Experiment 4 —Dog, 5 5 kgrm , heart weight 46 5 grm						
35 2° C	95	10	—	28	110	3 ·9
	95	40	—	22 5	106	4 7 11 per cent. CO_2
	95	10	—	26	106	4 ·0
	0 5 c.c. adrenalin, 1/10000					
	94	0	—	37 5	98	2 6
	94	40	—	26 ·5	100	3 8 20 per cent CO_2.
Experiment 5 —Dog, 3 kgrm., heart weight 33 5 grm						
33° C	130	330	—	15	85	
	1 c c adrenalin, 1/10000					
	130	330	—	24	77	3 2
	125	330	—	21	62	2 ·95 7 per cent CO_2 on

Experiments with Cats.—Various difficulties were encountered in making the experiments on cats, the main ones being due to the sensitiveness of the lungs. The blood of several cats had to be used in order to get sufficient to fill the tubes of the schema, and it seemed that the foreign blood was liable to cause œdema of the lungs, which soon brought the experiment to a conclusion. A typical example of the results obtained in a good experiment is given in Table IV, from which it will be seen that with various percentages of CO_2 no increase in the output per minute was obtained, while the heart rate is reduced considerably by the larger percentages In other experiments, using the suck and thrust pump devised

by Hans Meyer, and using a cardiometer attached to various forms of recorder, it was found that the output as measured was not increased, although in some instances the recorder showed an increased amplitude of stroke Since the output through the coronary system is not increased, the difference between these results and those of Jerusalem and Starling (4) probably lies in the inadequacy of the volume recorder used by them, which responded to the slower rhythm by a greater throw* On the other hand, cats are remarkably tolerant of CO_2 as compared with dogs, and it is of interest to note that, in the only experiment with dogs recorded by these authors, the output was much reduced by administering CO_2.

Adrenalin alone reduced the systemic output, but when adrenalin and CO_2 were used together the effective output was in some cases higher than in the normal preceding period

Table IV —Cat Heart weight, 12 grm

Temp	B P	I V C	Rate 10 secs	Output 10 secs	Output beat	
				c c	c c	
36° C	73	5	37	20	0 54	
	73	8	28	20	0·71	5·5 per cent CO_2
	72	4	36	21 5	0 58	
	72	10	30	20 4	0·68	10 5 per cent CO_2
	70	5	34	20 8	0 61	
	70	20	22	19·2	0·87	17 5 per cent. CO_2
34°	106	8	33	14	0·425	
	0 8 c c. adrenalin, 1/10000					
	100	18	47	7	0 149	
	105	24	45	13	0 29	
	104	18	40	13 1	0·32	17 per cent CO_2
	104	28	49	13	0·26	
	104	12	39	13	0·33	18 per cent CO_2
	105	20	46	13 5	0 29	
	106	15	34	14·3	0·42	20 per cent CO_2

Heart Rate—With all percentages of CO_2 in the inspired air there is a slowing of the heart rate, and this retardation becomes more marked as the percentage of CO_2 is increased The slowing sometimes lasts for a time after the CO_2 is taken off and ordinary air respired again.

Adrenalin causes, under all conditions, a quickening of the heart rate. With inhalation of CO_2 combined with the injection of adrenalin, the algebraical sum of the above effects is noticed. Sometimes during the

* Ketcham, King, and Hooker (11) found no increased output in the isolated cat's heart.

administration of CO_2 and adrenalin the ventricle drops to half its previous rate, due to heart block

Volume Changes in the Heart.—During the administration of CO_2 the mean volume of the heart is shifted towards the diastolic side, while adrenalin causes a diminution in the mean volume. This is shown in fig. 2 from curves obtained by the method described in a paper by Fühner and Starling (12), the protocols are given below

Table V—Mean Heart Volume.

Dog, 16·4 kgrm Heart, 128 grm

Time	Temperature	B P	I V.C.	Rate 10 secs
min secs				
–	35 5°	94	50	20
1 30	—	88	65	17 } 11 per cent CO_2
1 0	–	86	75	17 } 11 per cent CO_2
1 0	—	98	55	21
1 0	—	92	48	23
2 c c adrenalin, 1/100000				
1 0	—	94	42	35

During the administration of 11 per cent CO_2, this heart, weighing 128 grm, increased 50 c.c. in capacity

Fig 3 is taken from an experiment in which the cardiac volume was recorded by a plethysmograph attached to an Albrecht piston recorder of brass with vulcanite piston. It shows that the systolic volume is first increased, followed by increase in the diastolic volume during the same beat, the excursion being the same as before. The heart does not contract to its previous volume, but takes a new increased length. It may come to an equilibrium in the position of increased mean cardiac volume, but usually in our experiments the course shown in the figure is taken and the heart continues to dilate, the excursions become smaller as the systolic volume increases more rapidly than the diastolic volume, so that the output per beat is diminished and may even cease. The dilatation is greater, the larger the amount of CO_2 in the air breathed. When the CO_2 is removed and air respired, the heart resumes its normal mean volume gradually, and as the systolic volume now decreases more rapidly than the diastolic volume, the excursion of the recording lever (output per beat) is increased, and may for a time be above normal.

When 1 c.c. of a 1 in 10,000 solution of adrenalin was added to the blood in the experiment (Table V), bringing the concentration in the circulating

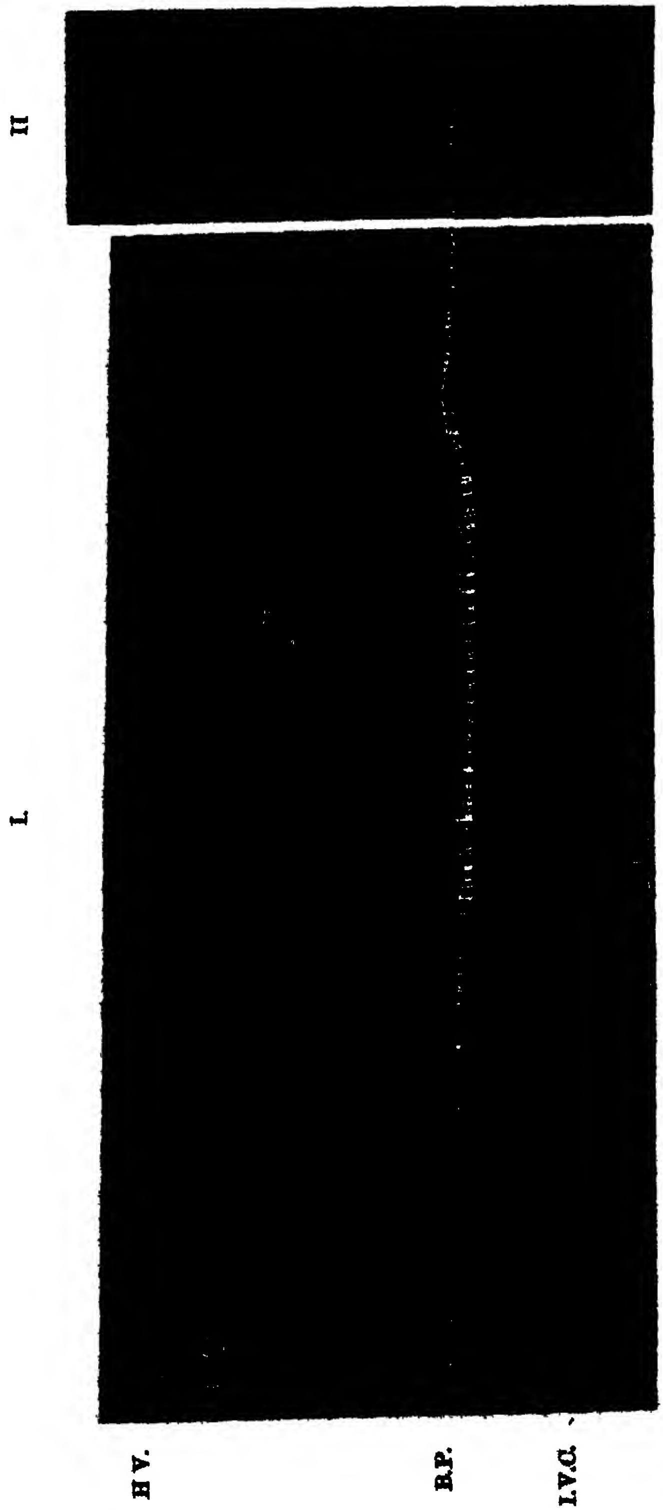

Fig. 2.—Effect of Carbon Dioxide (I) and Adrenalin (II) on Mean Heart Volume Read from left to right. Protocols in Table V Increase of cardiac volume caused fall of lever

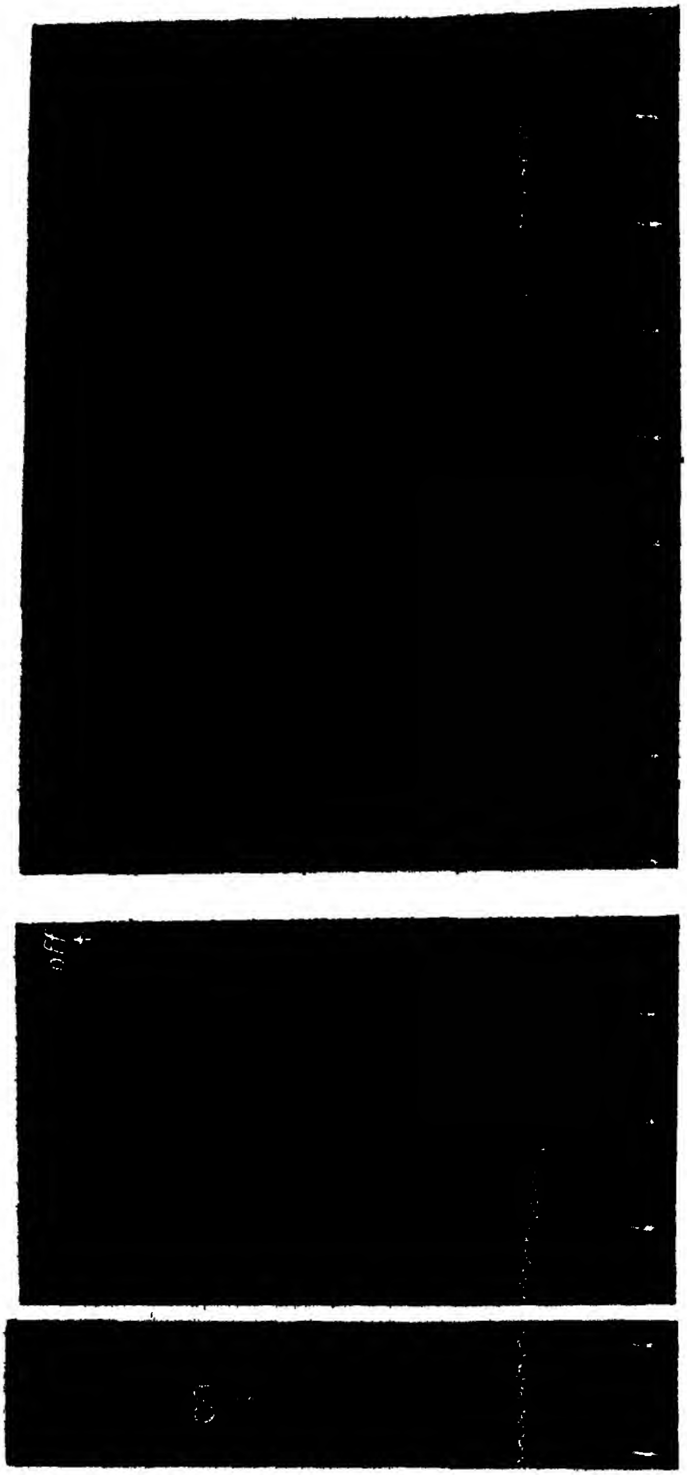

FIG 3.—Effect of CO_2 on Heart Volume and Blood Pressure. Read from left to right; Systole downwards; increase of cardiac volume caused rise of lever. Intervals 20 secs. Upper tracing, cardiometer; lower tracing, blood pressure.

blood to about 1 in 3,500,000, the mean heart volume diminished 17 5 c.c This takes place by the diastolic volume decreasing owing to lessened time between each beat for filling with the increased rate of the heart; and as the diastolic volume diminishes more rapidly than the systolic (fig 4) the output

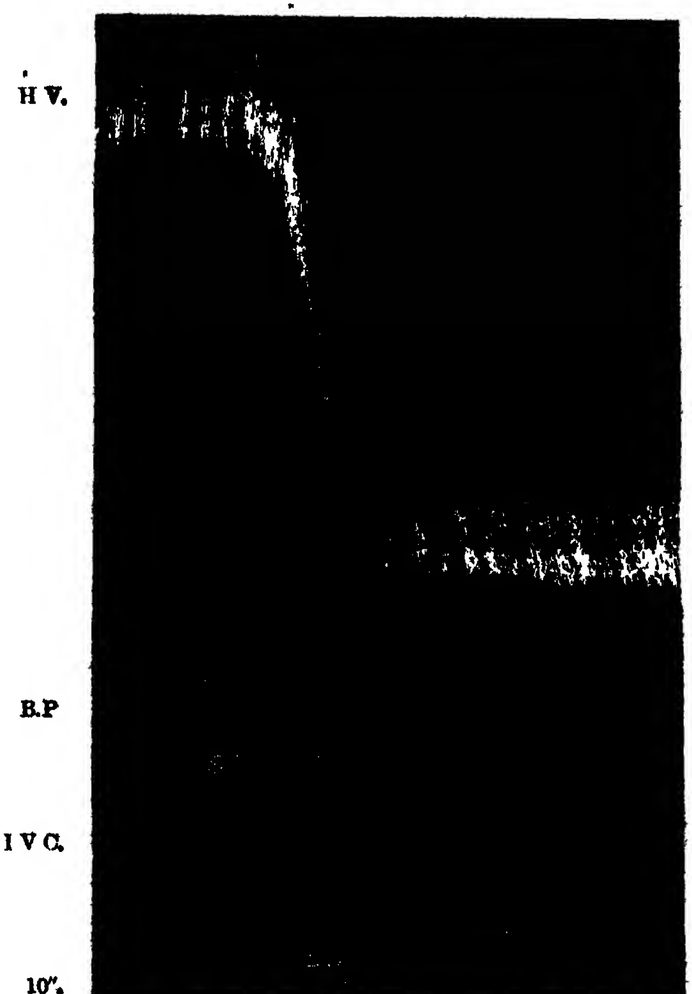

Fig. 4.—Effect of Adrenalin on Heart Volume, Blood Pressure, and Venous Pressure 0 1 mgrm. adrenalin added at the arrow Read from left to right. Systole downwards, decrease of cardiac volume caused fall of lever.

per beat is less. With recovery from adrenalin, the mean heart volume is greater than before its administration. A heart working badly and not dealing well with its venous inflow, so that the venous pressure is high, or a

heart working against a high arterial resistance, may dilate even in the presence of adrenalin.

Combining the administration of CO_2 and adrenalin in proper proportions may keep the mean heart volume constant, usually in a position shifted to the diastolic side, and the increased excursions of the lever (fig 5) correspond with the increased output per beat and output per minute described above.

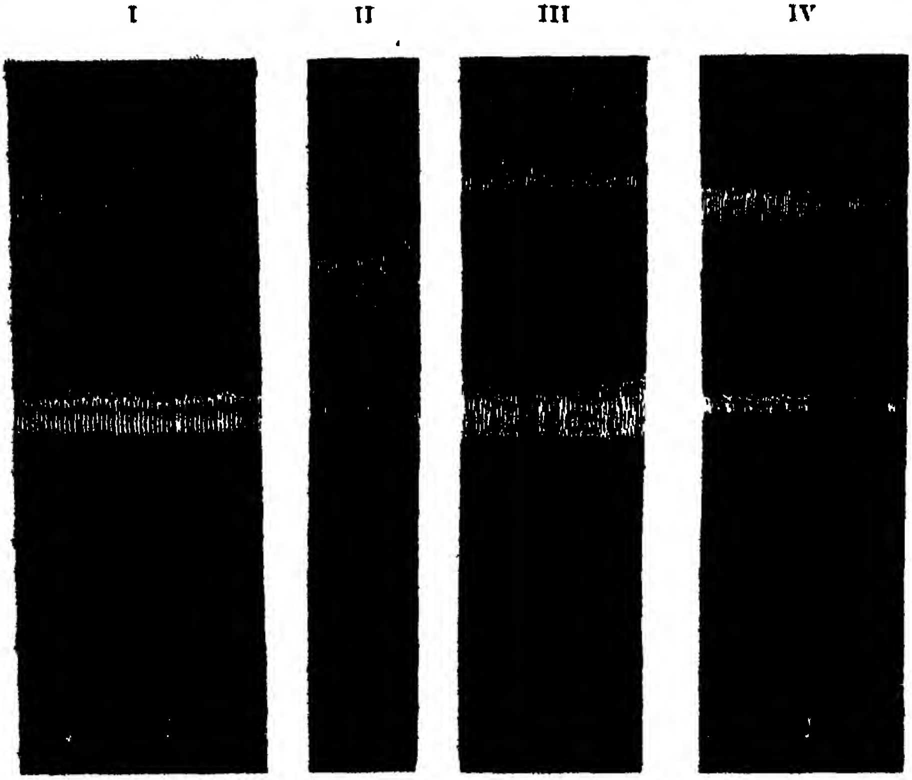

FIG. 5—Cardiac Volume and Blood Pressure, showing effect of combining CO_2 and Adrenalin (I) Normal, (II) adrenalin; (III) CO_2 and adrenalin, (IV) CO_2 off, recovery Read from left to right Intervals 2 mins Systole downwards, increase of heart volume caused rise of lever

Form of the Heart Volume Curves—These curves were obtained by means of a glass plethysmograph on the ventricles, the movements being carried by air transmission to the brass piston recorder with vulcanite piston made by Albrecht. The movements of the piston were arranged to write in a vertical plane and recorded on smoked paper (fig 6)

Normally, the form obtained by this method consists of a rounded curve, reaching its minimum usually with two points at which the curve changes to

I. II. IV

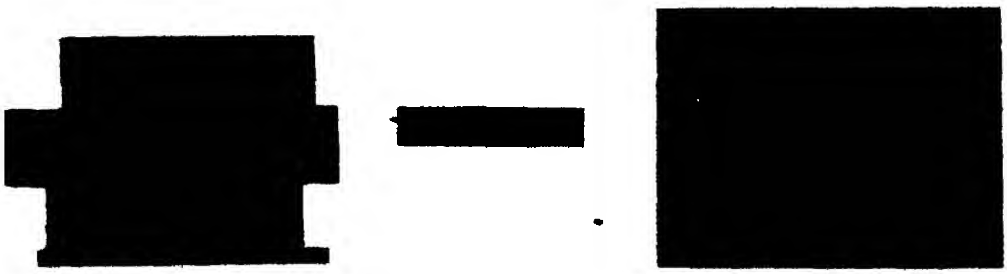

FIG. 6.—Ventricular Volume Curves. Read from left to right. Systole downwards Time 1/5 second (I) Medium venous inflow, normal, CO_2, adrenalin, (II) small venous inflow, adrenalin; (III) full venous inflow, adrenalin, adrenalin with CO_2.

be more convex to the abscissa, as the blood is driven out more slowly towards the end of systole. Diastole sets in rapidly and continues till, after about two-thirds of its course, it becomes less rapid, and then rises again parallel to its original curve to the maximum diastolic position, when the curve turns sharply into the downstroke of ventricular systole. With greater or smaller filling, the shape of the curve is steeper or more gradual, but only with hearts abnormally slowed by vagus stimulation is there a diastolic portion running nearly parallel with the abscissa, the part appearing to run parallel with the abscissa for an instant and interrupting the usual diastolic curve being probably due to the ventricles being drawn up slightly through the movable elastic diaphragm of the cardiometer by the contracting auricles.

When CO_2 is administered, the systolic downstroke is less steep, while the diastole is lengthened so that the next auricular systole occurs at the time that the heart volume has reached the maximum diastolic position.

With adrenalin the systolic downstroke is steeper than normal, and then reaches the minimum gradually, while the diastolic expansion is interrupted by the next auricular systole before the maximum dilatation is reached In many cases, with small venous inflow, the heart volume curve presents a flat top, the systole continuing after the ventricle has expelled the blood in it This is especially marked in curves obtained with small venous inflow after injection of adrenalin, when the major part of the output per beat is ejected in the first part of systole

With full venous supply after adrenalin the systolic and diastolic parts of the curve are smooth and rapid;* but when CO_2 is administered at the same

* The smoothness of the curve with adrenalin, however, is probably due to the period of the recorder being too slow, for using the recorder designed by Piper (14), in Berlin, which turns the volume changes into pressure changes and records these by a beam of

time the systole of the ventricle is slower, and the next auricular systole shows as a well marked interruption of the diastolic part

Endocardiac Pressure—The curves were obtained in Berlin on the heart-lung preparation by means of the small manometers designed by Piper (15) according to Frank's specifications, and recording photographically Figs 7 and 10 (1) show the effect on the form of the intraventricular pressure curve of

FIG. 7—Effect of 12 per cent CO_2 on Left Auricle and Ventricle Read from right to left Experiment 3, *a*, Table VI

(*a*) Normal, T 34 2, B P 102, I V C 12, O P 101 c.c in 10 secs

(*b*) 12 per cent CO_2, T 34 2, B P 102, I V C 30, O P '

small percentages of CO_2 in the air breathed, the isometric period is not so steep as in the normal curve, the ventricle taking a longer time to develop the same amount of pressure (see Table VI, 1, 2, 3, 5, 8) The total length of systole is longer, and relaxation is slower, but it is a delay of the whole process of the heart cycle, and the proportion of systole to diastole remains about the same as in the normal beat The maximum pressure exerted by the ventricle is usually a little less, 108 mm Hg, as against 117 mm in the normal, with the same venous supply and same arterial resistance (Table VI, Experiment 3)

After adrenalin the ventricle more rapidly reaches a certain tension in the isometric period,* and relaxation is also rapid, but the total diastolic time is proportionally longer than normal For instance, in Experiments 6 and 7, Table VI, the diastole represented 52 per cent of the total heart cycle,

light reflected from a mirror, we found the interruption due to auricular systole well marked, even with full venous supply after adrenalin.

* Wiggers (16) has already noted this in the right ventricle

Table VI.—Rate and Force of Contraction and Relaxation.

	Heart rate 10 secs.	As–Vs interval	Duration of heart cycle: Time.	Duration of heart cycle: Per cent.	Systole*: Time	Systole*: Per cent.	Diastole*: Time	Diastole*: Per cent.	Isometric period: Time	Isometric period: Pressure	Maximum pressure in ventricle
Experiment 1.—Dog, 7·5 kgrm., heart ?, cannulæ in left auricle and left ventricle.											
			sec.		sec.		sec.		sec.	mm.	mm. Hg
Normal	25	—	0·440	100	0·170	38·7	0·27	61·4	0·040	50	100
CO_2	21	—	0·490	100	0·188	38·5	0·302	61·5	0·047	50	102
CO_2 and adrenalin	34·5	—	0·285	100	0·090	31·6	0·195	68·4	0·013	50	80
Experiment 2.—Dog, 5·5 kgrm., heart 63 grm., cannulæ in left auricle and left ventricle.											
Normal	30	0·070	0·318	100	—	53·0	—	47·0	0·038	120	200
CO_2	25	0·065	0·398	100	—	49·0	—	51·0	0·041	120	235
Adrenalin	37	0·056	0·270	100	—	42·5	—	57·5	0·023	120	194
CO_2	26·5	0·091	0·376	100	—	47·0	—	53·0	0·041	120	220
CO_2 and adrenalin	36	0·062	0·277	100	—	39·0	—	61·0	0·029	120	225
Experiment 3.—Dog, 10·5 kgrm., heart 84 grm., cannulæ in left auricle and left ventricle.											
a { Normal	24	0·093	0·415	100	0·215	51·8	0·200	48·2	0·060	75	117
a { CO_2	22	0·093	0·455	100	0·243	53·5	0·212	46·5	0·060	75	108
Normal (V.S.+)	24	0·078	0·415	100	0·230	55·4	0·185	44·6	0·032	40	159
Adrenalin	37	0·068	0·272	100	0·107	39·4	0·165	60·6	0·015	40	156
Adrenalin	37	0·068	0·272	100	0·109	40·0	0·161	60·0	0·013	35	138
Adrenalin and CO_2	31	0·069	0·322	100	0·109	34·0	0·213	66·0	0·016	35	146
Experiment 4.—Dog, 8·5 kgrm., heart 66 grm., cannula in left ventricle and cardiometer.											
a { Normal	26	—	0·388	100	0·185	47·7	0·203	52·3	0·052	75	130
a { CO_2	25	—	0·400	100	0·195	48·8	0·205	51·2	0·056	75	130
b CO_2 and adrenalin	24·5	—	0·405	100	0·144	35·8	0·261	64·6	0·028	75	152
c { Adrenalin	31	—	0·320	100	0·118	36·8	0·208	63·1	0·022	75	141
c { Adrenalin and CO_2	26	—	0·400	100	0·135	33·8	0·265	66·2	0·028	75	170

Experiment 5 —Dog, 7 5 kgrm , heart 62 grm , cannula in right ventricle and cardiometer

a	Normal	28	—	0 355	100	0·142	40 0	0·213	60 0	0·042	15	25
	CO_2	26	—	0 480	100	0 160	42·0	0·220	58·0	0·046	15	16 4
b	Normal	26 5	—	0 377	100	0 138	36·6	0 239	63 5	0·042	15	17 2
	Adrenalin	43 5	—	0 228	100	0·090	40·0	0 187	60 5	0·020	15	34 5
c	Adrenalin and CO_2	40	—	0·250	100	0·089	35 5	0 161	64 5	0·022	15	39

Experiment 6 —Dog, 7 5 kgrm , heart 75 grm , cannulæ in left auricle and left ventricle

Normal	24 5	0 110	0 410	100	0 197	48	0 213	52	0·050	75	116
Adrenalin	38	0·063	0·262	100	0·097	37	0 165	63	0·014	75	314

Experiment 7 —Dog, 8 5 kgrm , heart 85 grm , cannulæ in left ventricle and left subclavian

Normal	28 5	—	0 550	100	0·257	46·8	0·293	53 2	0·063	65	126
Adrenalin	40	—	0 383	100	0 146	38 1	0 237	62	0·021	65	172

Experiment 8 —Dog, 9 5 kgrm , heart 70 grm , right ventricle and pulmonary artery

a	Normal	—	—	0 505	100	0 216	42 8	0 289	57 2	0·056	40	
	CO_2	—	—	0 538	100	0·283	53 5	0·250	46 5	0·064	40	
b	Normal	—	—	0 513	100	0·235	45 8	0 278	54·2	0·054	40	48
	Adrenalin	—	—	0 380	100	0 166	43 6	0·214	56 3	0·039	40	67 5
c	Adrenalin	—	—	0 380	100	0 146	38 4	0 234	61·0	0·0345	40	60
	Adrenalin and CO_2	—	—	0 415	100	0 169	40 7	0 246	59 3	0·046	40	63

* Systole = beginning of contraction to closure of aortic or pulmonary valves, diastole = remainder of heart cycle

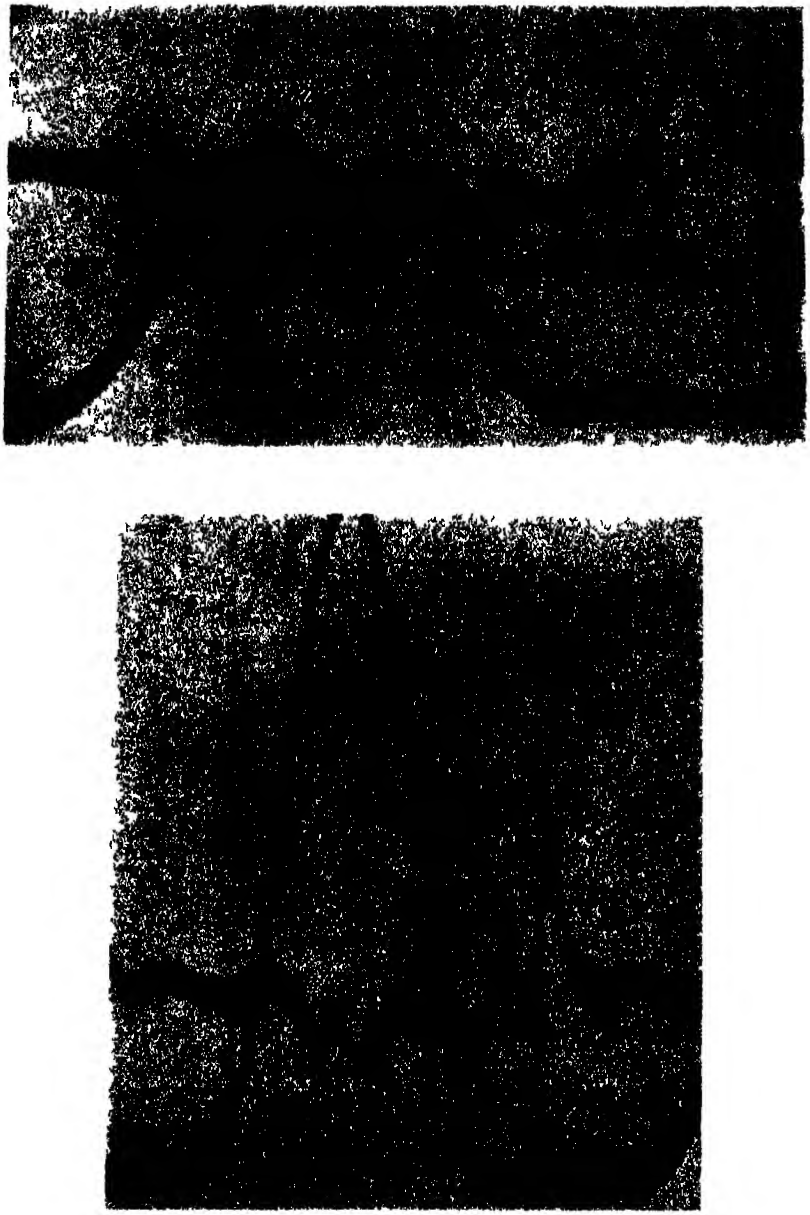

FIG 8 —Left Auricle and Ventricle before and after Adrenalin Read from right to left. Experiment 6, Table VI

(*a*) T 35 5, B P 84, I V C 20, O.P. 194 c c. in 10 secs., rate 24 5 in 10 secs (normal).
(*b*) T 36 4, B P 84, I V C 10, O P 194 c.c. in 10 secs., rate 38 in 10 secs. (after 0·1 mgrm. adrenalin).

and after injecting adrenalin, diastole was 63 per cent. of the total heart cycle. The shortening of the time of the contractile period is so great that, even with the increased rate of heart-beat, the heart is in a state of contraction for 3·7 seconds in 10 seconds, as against 4·8 seconds in 10 seconds in the preceding normal period. In the heart cooled by cooling the inflowing blood, the proportion of diastole to total cycle is 50 per cent. Evans and Ogawa (17) have found the metabolism of the heart to be increased by about three times after injecting adrenalin. Since the time per 10 seconds during which the heart is in a state of contraction is shorter than in the normal period, adrenalin seems to have a specific effect in mobilising the "contractile substance," or in exaggerating the changes taking place on the "active surface" of the muscle fibres during contraction. That the heart behaves in quite another manner after injecting adrenalin is shown in fig. 8, the protocols of which are given in Table VI, 6 and 7. They show the more rapid development of tension in the isometric period, the great rise of maximum pressure (116 to 314 mm. Hg) as the blood is shot violently out of the ventricle, and the relatively short duration of the whole contractile process. For a similar effect on the right ventricle, see fig. 10 (2), protocols in Table VI, 5.

When CO_2 and adrenalin are administered together, there is a slightly lengthened time to get up a certain tension in the isometric period, the maximum pressure in the ventricle attains a greater height than before, and there is marked lengthening of the diastolic period, thus allowing time for greater filling before the next systole begins [figs. 9 and 10 (3)].

Output per Beat.—During the inhalation of CO_2 in percentages from 3 to 11, the output per beat is equal to, or even greater than, before; but with percentages of 12–20, or more, the output per beat is diminished, and there may be no flow of blood at all. When the CO_2 is removed and ordinary air breathed again, the output per beat is greater than before the CO_2, since the efficiency of contraction of the heart recovers before the rate again becomes normal.

With adrenalin, the output per beat is diminished. Inhalation of CO_2 combined with addition of adrenalin, gives an output per beat, if the dosage is adjusted, equal to, or greater than, the normal (fig. 5).

As–Vs Interval.—Adrenalin shortens the *a–v* interval. Small percentages of CO_2 in the air breathed cause little or no alteration in the interval, but with larger percentages (above 12 per cent.) the interval is lengthened; when combined with adrenalin, CO_2 still has not much effect, but sometimes with large amounts of CO_2 the heart rate drops to half, and a 2 : 1 heart-block is set up. Lewis and Mathison (18) found heart-block to be of regular occurrence

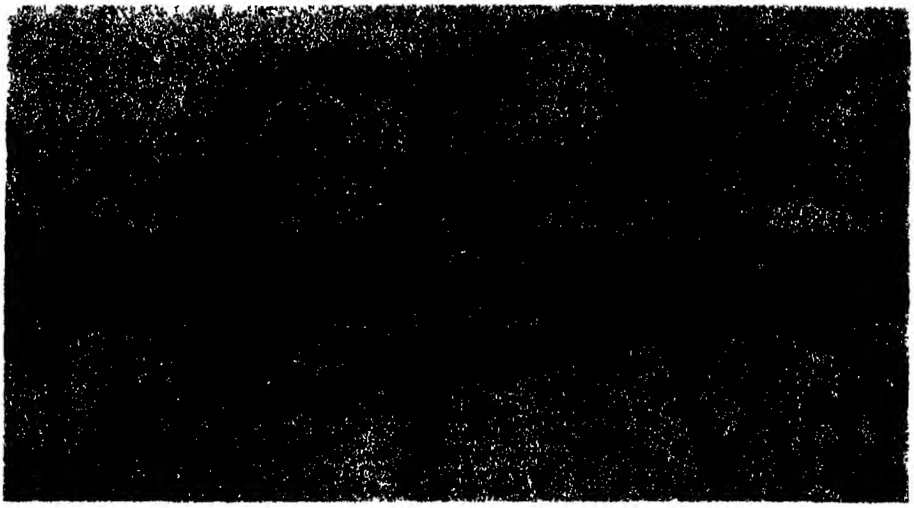

FIG 9.—Effect of Adrenalin, and Adrenalin combined with CO_2, on Left Auricle and Ventricle Read from left to right Experiment 2, Table VI

(*a*) T 35, B P 96, I V C 70, O P 100 c c in 10 secs (after adrenalin)
(*b*) T 35, B P 96, I V C 70, O P " (adrenalin and CO_2)

(1)

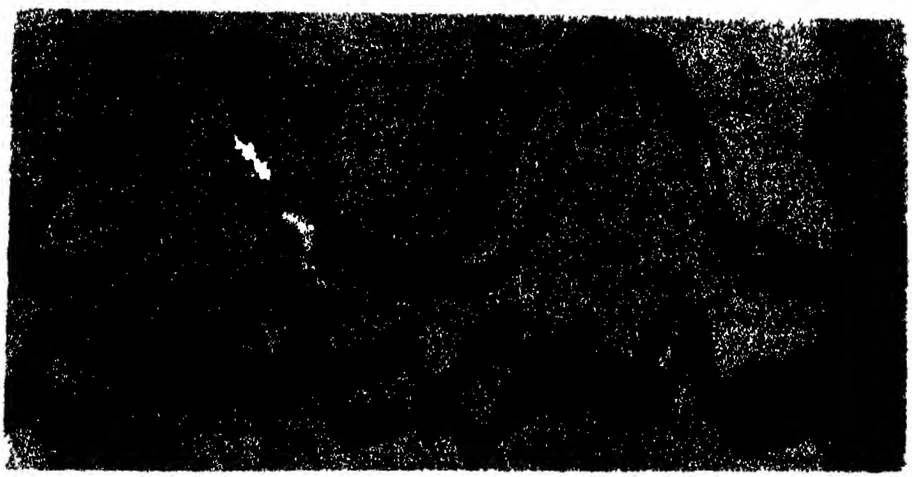

FIG 10 (1), (2), and (3).—Right Ventricle and Pulmonary Artery Read from right to left

Fig. 10 (1).—Effect of CO_2, Experiment 8, *a*, Table VI

(*a*) T 36 5, B P 92, I V C 22, O P 106 c c in 10 secs (normal)
(*b*) T 36 5, B P. 92, I V C 90, O P " (6 per cent CO_2)

(2)

Fig 10 (2). - Effect of adrenalin, Experiment 8, *b*, Table VI

(*a*) T 26 6, B.P 92, I V C. 38, O P 128 c c in 10 secs (normal)
(*b*) T 36 6, B P 92, I V C 12, O P ? (after 0 1 mgrm adrenalin)

(3)

Fig. 10 (3) – Adrenalin and CO_2, Experiment 8, *c*, Table VI

(*a*) T 36 6, B.P 92, I V C 12, O P ? (adrenalin alone)
(*b*) T 36 7, B P 96, I V C 22, O P 124 c c in 10 secs. (adrenalin with 6 per cent CO_2)

in asphyxia and independent of inhibition, and Mathison (19) found the cause to be due to lack of oxygen rather than excess of CO_2 in the spinal animal; but observed that heart-block may occur with large doses of CO_2 even in the presence of sufficient oxygen.

Summary and Discussion of Results

Recent work on contraction of skeletal muscle has tended to establish more and more the view that the phenomena of contraction can best be described by reference to alterations of the surface energy of the muscle elements, and the length of the muscle fibres is a measure of the surface of action. In the heart the mean volume is the guide we have to the length of the muscle fibres, and it has been shown (20) that the heart reacts to increased work by increasing its mean volume, whether the increased work is evoked by greater diastolic inflow or greater arterial resistance

It will be seen, from the results given above, that CO_2 in all doses appears to have a depressant action on the functions of the heart, the contractile stress is developed more slowly, the heart taking a greater mean volume to carry out its work, but if the CO_2 is continued, the observed output diminishes. Since the coronary circulation is unaltered this indicates a diminution of total ventricular output, and the venous pressure rises owing to the damming back of blood in the veins

Adding adrenalin to the blood circulating through a heart which is capable of responding, causes increased rate of contraction and rate of development of contractile stress; the heart can develop the requisite tension more easily and from a position of shorter initial length, so the mean heart volume is shifted to the systolic side. Adrenalin seems to have a specific action in mobilising the "contractile substance" and increasing the energy changes taking place at the surface of the muscle fibres during contraction. The result is that the ventricle contracts violently and the blood is expelled under great pressure into the aorta. The coronary perfusion is greatly increased and the nutrition of the heart improved. Relaxation takes place rapidly, and since there is no resistance to the inflowing blood the venous pressure falls, but the onset of the next systole comes so early that the filling of the heart, and consequently the output per beat, are less than normal, but the total output of the ventricle per minute is equal to or greater than normal.

With CO_2 and adrenalin combined in proper doses we still obtain greater rate of contraction and relaxation, but the whole diastolic period is lengthened; thus there is time for greater filling, and there is increased output per beat and per minute This increased observed systemic output

is accompanied by an increase in the coronary output, so that the total ventricular output is above normal The slower contraction is also more effective in driving a mass of blood into the aorta instead of firing it out suddenly.

Cannon (21) has recently summarised the evidence of the significance to the organism of the function of the adrenal medulla in times of great emergency We have found that the heart muscle is not only better nourished by increased coronary supply, but the contractile process is also strengthened in a specific manner We have probably obtained in the heart-lung schema with maximum venous inflow and proper proportions of CO_2 and adrenalin, the conditions occurring in short severe muscular exercise, where the muscles of the arms and abdomen are contracted, while the legs are active, all aiding the venous return to the chest, the increased depth of respiration also assisting the venous return. The small excess of CO_2 is both the call to the secretion of the adrenals, which dilates the coronary vessels and strengthens the cardiac contraction, and the cause of the lengthened diastole and time for greater filling, so that the maximum output of the heart can be obtained

Conclusions

1 Carbon dioxide alone depresses all the functions of the isolated heart.

2 Adrenalin, besides dilating the coronary vessels, has a specific action in increasing the rate and strength of ventricular contraction

3 The effect of carbon dioxide and adrenalin combined is still to allow of more rapid and stronger contraction and rapid relaxation, and also to lengthen the diastolic period Thus greater filling of the heart takes place and the heart is in a better condition for putting out a maximal output

I have much pleasure in recording my thanks to Prof Piper, of Berlin, for his assistance with the endocardiac pressure tracings, and to Prof Starling, of London, for the initiation and successful carrying out of the whole work

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The Influence of Salt-Concentration on Hæmolysis

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The question of the effect of salt-concentration on the phenomena involved in hæmolysis has already received a considerable amount of attention

Nolf originally showed that the presence of certain salts, in definite concentrations, inhibited hæmolysis, and his observations have been repeatedly confirmed

Markl, working with acid sodium phosphate, showed that the introduction of this salt into a hæmolytic mixture caused complete inhibition of hæmolysis when a certain concentration was reached He was also able to show that the presence of this salt did not prevent the combination of the antibody with the red cells. He therefore concluded that its action consisted in so influencing the osmotic relations of the cell membrane that the complement could not be fixed upon it. He found that this action was not specific for acid sodium phosphate, but could be observed with other salts, notably with hypertonic solutions of sodium chloride itself.

These results were confirmed by Ehrlich and Sachs, but these authors, interpreting their findings in the light of the side-chain theory, believe that the action of the increased saline concentration is produced by preventing the chemical union of the amboceptor and complement, and not by any change in the osmotic relations of the cell membrane

It is of interest to note that Muir and Browning have shown that the addition of sodium chloride to fresh serum, in quantities sufficient to inhibit the hæmolytic action of complement, also prevents the retention of the complement in the pores of a Berkefeld filter

The following investigations were undertaken in the hope of throwing further light on the part played by salt-concentration in preventing the union of red cells and complement

Attention has been confined throughout to the action of varying strengths of sodium chloride

Sheep corpuscles, three times washed, have been employed as the test red cells, and fresh guinea-pig serum as complement The inactivated serum of a rabbit, immunised by repeated intravenous injections against sheep's red cells, provided a powerful hæmolytic antibody

It seemed desirable to commence by repeating Markl's experiments, amplifying them in certain directions Without entering into details of the experiments performed, it may be said that Markl's conclusions were entirely confirmed, and that no action of the increased salt-concentration, other than the prevention of the combination of the complement with the sensitised red cells, could be demonstrated

Markl also showed, in the course of his experiments, that by adding increasing amounts of a hæmolytic serum it was possible to produce lysis in the presence of increasing amounts of acid sodium phosphate He, however, employed an active serum containing hæmolytic antibody and complement, and made no attempt to investigate these two factors separately An attempt was therefore made to estimate quantitatively how far the anti-hæmolytic action of the increased salt-concentration could be counteracted by increasing the concentration of the hæmolytic antibody or of the complement

The following experiment is a typical one, and the results obtained in it were repeatedly confirmed

Experiment—In this and similar tests, since it was desired to determine the increase in hæmolysis resulting from an increase in complement or antibody content consisting of a definite number of hæmolytic doses, the guinea-pig serum was first absorbed for 2 hours at 0° C with excess of sheep corpuscles, and a preliminary series of tests was put up to determine the minimal hæmolytic dose of this absorbed serum.

M.H D of Hæmolysin = 0·05 c.c. of a 1/250 dilution.
M H D of Complement = 0·03 c.c
Amount of Test Corpuscles = 0·05 c c of a 50-per-cent suspension

Hæmolysis in 1 hour at 37° C

M H D complement	1	1	1	1	1	2·5	5	10
M H D hæmolysin	1	2·5	5	10	100	1	1	1
Salt concentration— 0·8 per cent	Complete	Complete	Complete	Complete	Complete	Complete	Complete	Complete
1·0 „	Almost complete	„	„	„	„	„	„	„
1·2 „	Slight	Almost complete	„	„	„	Almost complete	Very marked	„
1·4 „	None	None	Almost complete	„	„	Slight	Moderate	Very marked
1·6 „	„	„	None	Almost complete		None	Slight	Moderate
1·8 „	„	„	„	None	„	„	None	Slight
2·0 „	„	„	„	„	„	„	„	None
2·2 „	„	„	„	„	Marked	„	„	„
2·4 „	„	„	„	„	Slight	„	„	„
2·6 „	„	„	„	„	Trace	„	„	„
2·8 „	„	„	„	„	None	„	„	„

NOTE — The two series are not strictly comparable, because, while it was always possible to add the doses of hæmolysin as 0·05 c c of a saline dilution, the amount of complement added necessarily varied The last series was the only one in which the amount added was sufficiently large to seriously influence the salt concentration, and here the values, instead of varying from 0·8 to 2·8 per cent., ranged approximately from 0·8 to 2·38 per cent In the series containing 100 M H D of hæmolysin, the tubes which showed little or no hæmolysis showed marked agglutination of the red cells; but this also decreased, and finally disappeared as the saline concentration increased

Having thus confirmed Markl's observation that increased saline concentration inhibits the combination of the complement with the sensitised red cells, and, further, that this inhibitory influence can, to a certain extent, be counterbalanced by increasing the amount of hæmolytic antibody present, it was natural to attempt to determine whether, in hypotonic solutions, the amount of antibody necessary to cause lysis would be decreased, and, finally, whether it was possible for complement to combine with red cells without the intervention of hæmolytic antibody in mixtures containing little or no ionisable salt

In considering the results obtained during this part of the investigation, it is necessary to bear in mind the action of hypotonic or salt-free media on complementary sera, especially the phenomenon known as complement-splitting.

An observation made by Sachs and Terruuchi in 1907, whilst studying the inactivation of complement in a salt-free medium, has an important bearing on the problem in hand. These observers found that guinea-pig serum alone was capable of causing a more or less marked degree of lysis of ox corpuscles in a salt-free medium, made isotonic by the addition of 7.8 per cent. saccharose, while the same serum had no lytic action in normal saline solution. They were not, however, prepared to admit that this phenomenon was really comparable to the hæmolysis which takes place in a mixture of red cells, hæmolytic antibody and complement. They found that, while the complement alone produced hæmolysis in saccharose solutions, but not in normal saline, complement acting together with a specific hæmolysin always produced more lysis in saline than in saccharose solution; and, in some cases, lysis was entirely absent when a complete hæmolytic system was allowed to act in a saccharose medium, but well marked when the same system interacted in normal saline solution. They noted, however, that inactivation for 30 minutes at 55° C. destroyed the power of the complement to produce this abnormal hæmolysis, and suggested that it might be due to "a peculiarity of the normal hæmolysin."

A large number of experiments were undertaken in order to further investigate this phenomenon. It is only necessary to present here the main results obtained; but it should be noted that different specimens of serum obtained from presumably normal guinea-pigs gave very varying results, while a single given specimen often showed marked changes within 24 to 48 hours though stored on ice.

The specimens of serum vary in two ways, in their power of inducing lysis in salt-free media and in their resistance to the anti-complementary action of such media; and these two variations do not run parallel. Thus, a given specimen of serum may be actively lytic in a salt-free medium, but be easily inactivated by this same medium in the absence of red cells, while another specimen may be feebly lytic but suffer only a slight degree of inactivation, a third being actively lytic and markedly resistant to the inactivating action of the salt-free medium.

The specimens of guinea-pig serum used in these experiments were absorbed at 0° C. with sheep's red cells for a period varying in different experiments from one to two hours, in order to remove any trace of normal hæmolysin that might be present. It was found that the great majority of sera so treated

had a definite lytic action on sheep corpuscles when allowed to act on them for one hour at 37° C in a salt-free medium, the hæmolysis varying with different sera from the merest trace to complete solution

A few sera which, when untreated, produced hæmolysis, failed to do so after the preliminary absorption, while a few others produced no lysis before or after treatment The lysis was never very rapid, thus in no case was complete solution ever observed in less than 30 minutes

Inactivation at 55° C for 30 minutes completely destroyed the activity of every specimen of serum examined in this way

In a preliminary series of experiments fresh guinea-pig serum was absorbed with sheep corpuscles, and then varying amounts were added to 0 05 c c of a 50-per-cent suspension of sheep's red cells contained in 1 c c of 7 8-per-cent saccharose solution The following results may be taken as typical —

Experiment—A series of tubes was put up, each containing 1 c c of 7·8-per-cent saccharose solution and 0·05 c c of a 50-per-cent suspension of sheep corpuscles Varying amounts of the complementary serum were then added and the whole series incubated at 37° C for one hour A control tube, containing the same amount of red cells suspended in 7 8-per-cent saccharose solution to which had been added 0·8 per cent of sodium chloride,* and the maximum amount of complement employed, was put up, also a similar tube with the addition of 4 M H D. of hæmolysin

Amount of complement added to salt free tubes	Hæmolysis in 1 hour at 37° C
c c	
0 0	None
0 05	Moderate
0·075	Marked
0·1	Slight
0 125	Trace
0 15	None
0 175	"
0 2	"
0 225	"
0 25	"

Controls	Hæmolysis in 1 hour at 37° C
In 1 c c of 7 8 per-cent saccharose containing 0·8 per cent sodium chloride —	
(1) 0 05 c c red cells+0 25 c c complement	No hæmolysis
(2) 0 05 c c red cells+0 25 c c complement+4 M H D hæmolysin	Complete hæmolysis

* This medium is hereafter referred to as "normal saline-saccharose solution."

It is clear from this experiment that only a certain definite amount of complement will cause hæmolysis, an increase or decrease in this amount leads to a rapid reduction and final disappearance of the lytic action

These results are wholly unlike those obtained when working with a complete hæmolytic system reacting in normal saline solution It seemed possible that they might be explained by the fact that in increasing the amount of complementary serum we necessarily increase the amount of electrolytes, and that hence we might pass the limit at which complement could be absorbed by the red cells in the absence of a hæmolytic antibody Experiments carried out to test the validity of this explanation gave very definite results

Experiment—A series of tubes was put up, each of which contained 1 c c of 7 8-per-cent saccharose and 0 05 c c of a 50-per-cent suspension of washed sheep corpuscles After the addition of the constituents mentioned below, the whole series was incubated for one hour at 37° C

Tube		Hæmolysis in 1 hour at 37° C
	Each tube contained 1 c c of 7 8 per cent saccharose solution + 0 ·05 c c red cells	
1	+0 ·05 c c complement	Almost complete
2	+0 10 "	None
3	+0 25 "	"
4	+0 05 " +0 ·05 c c 0 8 per cent saline	Slight trace
5	+0 ·05 " +0 20 " 0 8 "	None
6*	+0 ·05 " +0 ·05 " hæmolysin	Complete
7	+0 05 " +0 20 " "	"
8	+0 ·05 c c complement + 0 ·05 c c hæmolysin + 0 ·05 c c inactivated complement	None

* The hæmolysin was diluted with normal saline so that 0 05 c c contained 2 M H D

This experiment shows that it is the raised salt concentration which prevents the hæmolysis in the tubes containing the higher quantities of complementary serum Thus, 0 05 c c of complement causes almost complete lysis while 0 25 c c causes none. But 0 05 c c of complement + 0 20 c c of 0 8-per-cent saline also produce no lysis

It will be noted also that in this experiment the addition of a hæmolytic serum causes increased lysis, but if we also add 0 05 c c of the same serum which was employed as complement, previously heating it at 55° C for 30 minutes, no lysis results Here, again, the effect of increased saline concentration in combating hæmolysis is clearly seen

These results help to explain those obtained by Sachs and Terruuchi, which led those observers to deny the identity of the hæmolysis produced

by complement alone in salt-free media with that produced by a specific hæmolysin in normal saline.

The dilution of a complementary serum with 10 times its volume of 7·8-per-cent saccharose solution, and the subsequent incubation of the mixture for one hour at 37° C., destroys the activity of the complement, but, if we add 0·075 c.c. of complement to 1 c.c. of 7·8-per-cent. saccharose solution, containing 0·05 c.c. of a 50-per-cent. suspension of sheep corpuscles, and then incubate at 37° C. for one hour, we shall obtain a more or less marked degree of hæmolysis. This can only mean that the complement which has become attached to the red cells is no longer subject to the destructive action of the salt-free medium. Thus, it is clear that the addition of a hæmolytic serum will only tend to increase hæmolysis, if the increased tendency to combination of red cells and complement, caused by the hæmolytic antibody it contains, is greater than the decreased tendency to combination resulting from the increased saline concentration. For unless combination is fairly rapid the destructive action of the markedly hypotonic medium on the complement will come into play. It follows that we should expect very powerful hæmolytic sera to increase lysis under these conditions, and weak ones to decrease it.

In general, the active sera, which are obtained by immunising rabbits against sheep corpuscles, tend to markedly increase hæmolysis in saccharose solutions, but this is not always the case, and in some experiments it was found that more lysis occurred with complement alone than with complement and hæmolysin, thus confirming the observation of Sachs and Terruuchi. The addition of serum, or serum diluted with saline, seems always to act more powerfully in inhibiting hæmolysis in salt-free media than does the addition of an equal amount of normal saline solution alone, and, although the hæmolytic sera here employed were always greatly diluted, it is possible that this factor came into play.

Keeping these facts in mind we may pass to the consideration of experiments in which varying amounts of hæmolysin were allowed to act in varying strengths of saline in 7·8-per-cent. saccharose solution, in the presence of 0·05 c.c. of a 50-per-cent. suspension of sheep corpuscles and 0·05 c.c. of complementary serum.

Experiment.—Each tube contained 1 c.c. of 7·8-per-cent. saccharose solution to which had been added varying amounts of sodium chloride. To each tube was added 0·05 c.c. of a 50-per-cent. suspension of sheep corpuscles and 0·05 c.c. complement. To each tube was then further added the amount of hæmolysin indicated in the left-hand column. This hæmolysin was added as 0·05 c.c. of a dilution of the required strength in the saline-saccharose solution. Incubation was carried out for 1 hour at 37° C.

Hæmolysis

Hæmolysin in M H D	Percentages of sodium chloride in the saccharose solution										
	0·8	0·72	0·64	0·56	0·5	0·44	0·38	0·32	0·26	0·2	0·0
0	None	None	None	None	None	None	None	None	None	None	Moderate
0·1	Trace	Slight	Slight	Moderate	Moderate	Moderate	Slight	Trace	„	„	Slight
0·25	Marked	Marked	Marked	Very marked	Marked	Marked	„	Slight	„	„	„
0·50	„	Almost complete	Almost complete	Almost complete	Complete	Almost complete	Very marked	Moderate	Slight	Slight trace	„
1·0	Complete	„	Complete	Complete	„	Complete	Complete	Complete	Marked	Moderate	Moderate
5·0	„	Complete	„	„	„	„	„	„	Complete	Almost complete	Complete
10·0	„	„	„	„	„	„	„	„	„	Marked	„

From this experiment it is clear that —

(1) When no hæmolytic antibody is added, hæmolysis only occurs in the tube which contains no salt, except the small quantity present in the 0 05 c c of guinea-pig serum employed as complement

(2) When amounts of hæmolytic antibody are added below the minimal hæmolytic dose (previously determined for hæmolysis occurring in normal saline-saccharose solution), the degree of lysis at first increases as the salt concentration decreases. For instance, while 0 1-M H D produces only a trace of hæmolysis in 0 8-per-cent saline it produces moderate hæmolysis in the tubes containing 0 56 per cent to 0 44 per cent sodium chloride, and, while 0 5 M H D produces marked hæmolysis in the presence of 0·8 per cent sodium chloride, it produces complete hæmolysis in the 0 5-per-cent solution

(3) When, however, the lower concentrations of saline are reached, the anti-complementary factor comes into play and the hæmolysis again decreases. The hæmolysis in almost all cases reaches its minimum in the tube containing 0 2 per cent sodium chloride, probably for the reason that this amount of salt is sufficient to prevent or delay the union of red cells and complement, unless a considerable amount of hæmolytic antibody is present, while the hypotonicity is sufficiently marked to rapidly destroy the activity of the complement

It will be noticed that, although the hæmolysis in the great majority of the tubes presents an ordered variation, certain tubes show irregular results, in the above experiment, for instance, the tube containing 1 M H.D in 0 72-per-cent saline and that containing 10 M H D in 0 2-per-cent. saline.

Several similar experiments, however, all yielded results agreeing in all substantial particulars with those indicated above, though in all there were individual tubes which showed irregular hæmolysis One factor which accounts for this is that the cells tend to sink rather rapidly in the saccharose solution, and sometimes undergo agglutination, while in a few cases the corpuscles show marked agglutination in the saccharose solution before any serum is added, though they remain evenly distributed in control saline tubes

Having established the fact that, in passing from markedly hypertonic to markedly hypotonic saline solutions, less and less hæmolytic antibody is needed to bring about the union of red cells and complement, and that, when a completely salt-free medium is reached, this union takes place unaided, it seemed necessary to enquire further into the nature of this direct or unaided combination

We know that when dealing with a mixture of red cells, hæmolytic antibody, and complement, reacting in a medium of normal saline solution,

the red cells and antibody combine at a temperature of 0° C, while the further combination of complement with the red-cell-antibody complex only occurs when the temperature is raised

An attempt was made to determine whether the combination of red cells and complement in a salt-free medium conformed to this rule, as we should expect if the union were really a direct or unaided one This observation is of considerable importance, since it would obviously be theoretically possible for an antibody to be involved, which was present in the guinea-pig serum, but which only acted in salt-free media, and which therefore was not removed when the undiluted serum was absorbed on ice with sheep corpuscles.

The following experiment yields a definite indication as regards this possibility —

Experiment—A number of tubes were prepared, each containing 1 c c of a 7 8-per-cent saccharose solution To one, red cells and complement were added and incubation was carried out for one hour at 37° C. This tube was controlled by another containing the red cells and complement in 1 c c. of the normal saline-saccharose solution To further tubes of saccharose solution were added in some cases red cells and complement, and in others complement alone, and these tubes were kept for one hour at 0° C Some of these tubes then rapidly centrifugalised, and the supernatant fluid and deposit treated as indicated

The red cells were added as 0 05 c c. of a 50-per-cent suspension in saccharose solution, 0 075 c c of complementary serum was added in every case

	Hæmolysis
Incubation for 1 hour at 37° C —	
(1) Complement + red cells in saccharose	Very marked
(2) „ „ „ — 0 8 [illegible]	None
1 hour at 0° C followed by incubation for 1 [illegible] following treatment—	
(3) Tube containing red cells + complement shaken	Very marked
(4) Deposit from one (red cells + complement) tube + supernatant fluid from another	„ „
(5) Supernatant fluid from one (red cells + complement) tube + fresh red cells	„ „
(6) Deposit from one (red cells + complement) tube + 1 c c of saccharose solution	None
(7) Supernatant fluid from a tube containing complement alone + fresh red cells	Very marked
(8) A tube containing complement alone shaken + red cells	„ „

From these results it follows that —

(1) The red cells do not absorb the whole complement at 0° C, since when they are separated by centrifugalisation and, after being suspended in fresh

saccharose solution, are incubated for one hour at 37° C., they undergo no hæmolysis.

(2) No antibody capable of combining with red cells at 0° C is involved, since the supernatant fluid after one hour's absorption on ice is capable of hæmolysing fresh red cells.

It is important to note that in this experiment the complement is not appreciably affected by the dilution with salt-free saccharose solution at 0° C., since when a tube containing this mixture was centrifugalised the supernatant fluid was capable of hæmolysing added red cells on further incubation at 37° C

A somewhat different result was, however, often obtained While the deposit from a tube containing red cells and complement in saccharose solution, which had been kept for one hour at 0° C, never showed any trace of hæmolysis when suspended in fresh saccharose solution and incubated for a second hour at 37° C., it frequently happened that the supernatant fluid failed to produce more than a trace of hæmolysis when incubated at 37° C with fresh corpuscles In such cases it was always found that, if the tube containing complement alone in saccharose solution was similarly kept for one hour at 0° C, then centrifugalised and the supernatant fluid added to fresh red cells, only a trace of hæmolysis occurred upon further incubation at 37° C It is therefore clear that the failure of lysis is due to the action of the salt-free medium on the complementary serum, and not to any fixation of the complement by the red cells in these cases At the same time, it was found that if the supernatant fluid from one of these tubes containing complement only was added to the deposit from a tube which, during the preliminary treatment at 0° C, had contained both red cells and complement, then a degree of lysis resulted during the second hour's incubation at 37° C, which corresponded with the hæmolysis which occurred when a tube containing red cells and complement in saccharose was incubated at 37° C, or kept on ice for one hour and then shaken and incubated for another hour at 37° C

This clearly indicates that some part of the serum has been precipitated and is present with the red cells in the deposit In other words a certain degree of complement-splitting has taken place.

From other experiments performed during this part of the investigation this does not seem to be the whole explanation. It was found in many cases that, when a tube containing complement alone in saccharose solution was kept on ice for one hour and then well shaken, the contents failed to hæmolyse fresh red cells, though still producing lysis of the deposit from an iced saccharose tube containing red cells and complement. It seems clear,

therefore, that the presence of the red cells prevents the action of the salt-free medium on the complementary serum from progressing beyond a certain point. Experiments carried out to test the hypothesis that this might be due to a combination of the mid-piece with the red cells gave no definite results, for reasons which need not be entered into here

The fact remains that those complementary sera which are unaffected by simple dilution with salt-free saccharose solution at 0° C show no evidence of any absorption of the complement by red cells at this temperature We are, therefore, justified in saying that complement, combining directly with red cells in a salt-free medium, behaves in the same manner as complement combining with red cells in a medium of normal saline solution under the influence of a hæmolytic antibody, in that no combination occurs at a temperature of 0° C

In the experiments described above, and in the discussion of the results obtained, the terms "complement" and "hæmolytic antibody" have been employed in their usually accepted sense It is obvious, however, that the sera employed contain many other substances, and the recent cross-absorption experiments of Thiele and Embleton show that a hard and fast division into complement and antibody is not permissible It is, therefore, possible that not the whole complement, but only some specialised part of it can combine, unaided, with red cells in the absence of electrolytes, but this consideration does not affect the main thesis that a combination, that is impossible in a salt-containing solution without the addition of a special antibody, can occur in its absence in a salt-free medium.

Conclusions.

In the case of the hæmolysis of sheep corpuscles by guinea-pig complement, it is found that —

1. The presence of an excess of an electrolyte (sodium chloride) above the normal limit in a hæmolytic mixture prevents the combination of the complement with the red-cell-antibody complex

2 If the concentration of the antibody be markedly increased, it is possible, up to a certain point, to counteract the effect of the increased salt concentration.

3 If the salt concentration be decreased, a decreasing concentration of antibody serves to produce the union of red cells and complement

4. In an almost completely salt-free medium the combination occurs in the complete absence of antibody.

It only remains for me to record my great indebtedness to Dr. S. G. Platts for the assistance which he has rendered me throughout a considerable part of this investigation

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The Influence of the Hydrogen Concentration upon the Optimum Temperature of a Ferment.

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(Communicated by Prof W Bulloch, F R.S Received December 17, 1914.)

The present investigation is an outcome of previous work,* resulting in the discovery that the optimum temperature of any ferment, or ferment function, occurring in a given enzymic preparation, is independent of the concentration both of the substrate and of the enzyme, the duration of the action being constant. To follow up this observation it was felt desirable to investigate in a similar way the influence, if any, of the reaction, that is of the hydrogen ion concentration of the medium, on the optimum temperature of enzyme action the more so because enzymes, as regards their activity, are known to be extremely sensitive to this factor—some requiring an acid, others a neutral, and others again an alkaline medium in which to act. The question, moreover, is of special interest on account of the fact that Sorensen, in his classical researches on the *rôle* of the ionic concentration of the medium in activating enzymes, alludes to it, and predicts in regard to it that, when investigated, the optimum temperature will no doubt be found to vary with the hydrogen ion concentration of the medium.† That

* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 87, p. 245 (1914), B, vol. 88, p. 258 (1914).

† S. P L. Sörensen, 'Comptes Rendus des Travaux du Laboratoire de Carlsberg,' vol. 8, p. 146 (1909).

opinion, being of a speculative nature, was not deemed a sufficient answer to the question, its experimental investigation therefore became the more needed

The enzyme chosen was the *maltase* of *Aspergillus oryzæ*, the same preparation being used as had already been studied in a former communication,* where its optimum temperature, in an action of 16 hours' duration, and H^+ concentration that of the preparation simply dissolved in redistilled water, is shown to be $+47°$

Two stages occur in the investigation (1) A determination of the optimum reaction curve of the enzyme in an action of 16 hours' duration at $+47°$, for chosen dilutions of substrate and of enzyme, in presence of progressively increasing quantities of acid and of alkali added to the reaction mixture, (2) separate determinations of the optimum temperature of the ferment under the same conditions of substrate concentration, of enzyme concentration, and of duration of the experiment, for different hydrogen ion concentrations of the medium, corresponding to various points on the above optimum reaction curve

The substrate concentration chosen was M/20, or 18×10^{-3} grm of hydrated maltose per cubic centimetre of the reaction mixture, and the enzyme concentration was 6×10^{-4} grm of the enzyme preparation in powder per cubic centimetre of the reaction mixture

For the determination of the optimum reaction curve the practical details were as follows —First, a solution of the enzyme was prepared by dissolving 30 mgrm of the preparation in 10 cm.³ of redistilled water A clear pale amber-coloured solution resulted, which, after standing from a half to one hour at the ordinary temperature, was introduced in portions of 1 cm.³ into a series of eight clean test-tubes (Fischer's extra resistance glass) already containing 90 mgrm of hydrated maltose and either 4 cm.³ of pure water or a solution containing a known quantity of acid or alkali The tubes, after closing with clean sterile corks, were plunged into a water bath regulated at $+47°$ After 16 hours' incubation they were withdrawn, the corks removed, and each rapidly washed with 1 cm.³ of water—the washings being carefully added to the contents of the corresponding tube. Next, the tubes were heated for seven minutes in boiling water to stop the enzyme action, after which they were cooled and the contents diluted to 50 cm.³. The proportion of maltose hydrolysed was then determined, by Bertrand's method,† on 20 cm.³ of the diluted mixture The numbers obtained are set out in Table I.

* *Ibid.*

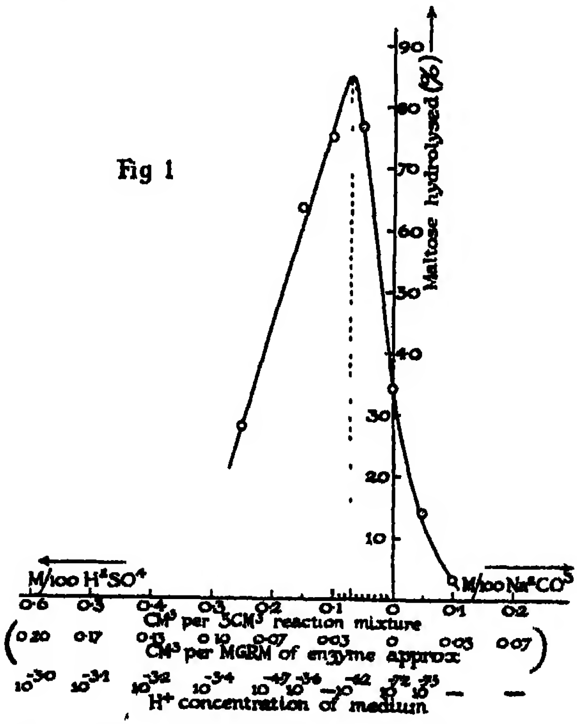
† 'Bull. Soc. Chim.,' (3), vol. 35, p. 1285 (1906).

Table I.

Quantities of acid and alkali added per 5 cm.³ of the reaction mixture	Maltose hydrolysed
	Per cent
0 25 cm.³ M/100 H_2SO_4	28 2
0·15 " "	68 5
0 10 " "	75 1
0·05 "	76 8
0 00 " (natural reaction, control)	84 3
0·05 " M/100 Na_2CO_3	14 2
0 10 " "	3 2

When the percentage of maltose hydrolysed is plotted against the quantities of reagents added, the curve indicated in fig 1 is obtained

Fig 1



This curve shows that under the conditions of the experiment the addition of the merest trace of alkali is detrimental to the enzyme action, while on the contrary the addition of acid increases the efficiency of the enzyme, until a certain point is reached, corresponding to the presence of 0·07 cm.³ M/100 H_2SO_4 in the 5 cm.³ reaction mixture, beyond which the addition of more acid is in turn detrimental to the action. In other words, the action passes by a maximum situated in the acid region

Measurement of the hydrogen ion concentration of the medium, resulting from the addition of various quantities of acid and of alkali per 5 cm.^{3} of the reaction mixture, containing 3 mgrm. of dissolved enzyme, were made by the colorimetric method of Sörensen (*loc cit*) The results are contained in Table II, and for convenience of description, in what follows, they have been reproduced on the base line of fig 1, underneath the respective quantities of M/100 H_2SO_4 and M/100 Na_2CO_3 which give rise to them

Table II

Quantities of acid and of alkali added per 3 mgrm of enzyme contained in 5 cm.^{3} of the reaction mixture		Corresponding H^+ concentrations
0·60 M/100 H_2SO_4	H^+ concentrations greater than the optimum of fig 1	$10^{-3.0}$; citrate, methyl orange
0·40 "		$10^{-3.2}$, " "
0·30 "		$10^{-3.4}$; " "
0·20 "		$10^{-4.7}$; phosphate, neutral red
0·15 "		$10^{-5.6}$; " "
0·07 "	H^+ concentrations equal to the optimum of fig 1	$10^{-6.2}$, " "
0·04 "	H^+ concentrations less than the optimum of fig 1	$10^{-6.8}$; " "
0·00 (natural reaction)		$10^{-7.2}$; " "
0·05 M/100 Na_2CO_3		$10^{-7.4}$, "

The second stage of the enquiry, which consists in determining the optimum temperature of the enzyme for a series of hydrogen ion concentrations corresponding to different points on the optimum reaction curve, figured in fig 1, was next undertaken Nine different H^+ concentrations of the medium were thus studied $10^{-3.0}$, $10^{-3.2}$, $10^{-3.4}$, $10^{-4.7}$, $10^{-5.6}$, $10^{-6.2}$, $10^{-6.8}$, $10^{-7.2}$, and $10^{-7.5}$

The practical details of the first determination in the series may be given as an example, to show how these optimum temperature determinations were carried out A solution of the enzyme was prepared containing 3 mgrm of the preparation per cubic centimetre and, after standing for a half to one hour, was introduced in portions of 1 cm.^{3} into eight clean test-tubes, already containing 90 mgrm. of maltose, 0·6 cm.^{3} of M/100 H_2SO_4 and 3·4 cm.^{3} of redistilled water. Such a mixture, according to Table II (or fig 1), corresponds to a H^+ concentration of $10^{-3.0}$ The tubes were closed with sterile corks, plunged into water thermostats at known temperatures, and incubated for 16 hours, when the enzyme action was stopped and the quantity of maltose hydrolysed in each tube determined as before. The numbers found are set out in Table III, together with the numbers obtained for the other members of the series.

Table III.

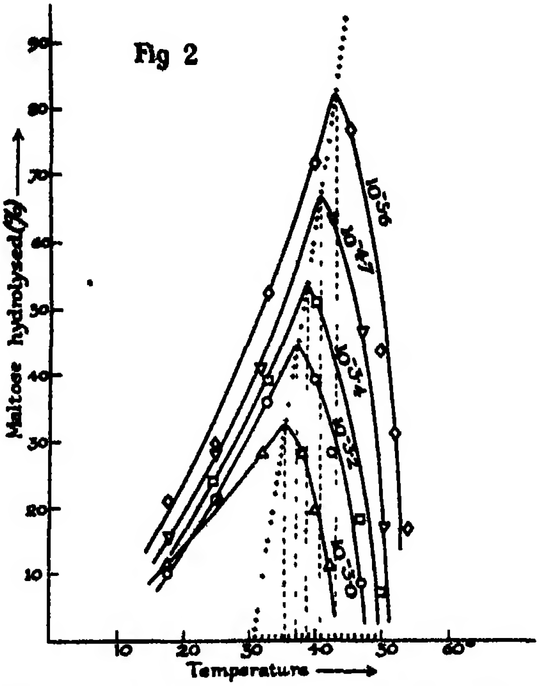
Temperatures at the beginning and end of each experiment	Maltose hydrolysed per cent for the following H^+ concentrations of the medium								
	$10^{-3.0}$	$10^{-3.2}$	$10^{-3.4}$	$10^{-4.7}$	$10^{-5.6}$	$10^{-6.1}$	$10^{-6.8}$	$10^{-7.2}$	$10^{-7.6}$
17·6–17·7°	—	—	—	—	21·1	—	—	—	3·2
17·7–17·8	11·4	10·0	—	—	—	—	—	—	—
18·0	—	—	15·5	—	—	—	—	—	—
23·0	—	—	—	—	—	26·8	—	7·2	—
24·5–24·6	—	—	24·0	—	—	—	—	—	—
24·8–25·0	—	—	—	28·2	29·7	—	—	—	8·6
25·0–25·1	—	21·1	—	—	—	—	16·9	—	—
25·2–25·4	21·1	—	—	—	—	—	—	—	—
27·8–27·9	—	—	—	—	—	35·9	—	12·8	—
32·0–31·6	—	—	—	40·6	—	—	—	—	—
32·2–32·1	28·2	—	—	—	—	—	—	—	—
32·8	—	35·9	39·1	—	52·5	—	32·8	—	12·8
34·5–34·6	—	—	—	—	—	57·1	—	21·1	—
37·8–38·2	28·2	—	—	—	—	—	—	—	—
40·0	19·7	39·1	50·9	—	71·7	—	52·5	—	15·5
41·8	—	—	—	—	—	78·5	—	28·3	—
42·3–42·2	11·4	—	—	—	—	—	—	—	—
42·4–42·5	—	28·2	—	—	—	—	—	—	—
42·5–42·6	—	—	—	63·5	—	—	—	—	—
45·4	—	—	—	—	76·8	—	—	—	21·1
46·8–47·0	—	8·6	18·3	—	—	—	—	—	—
47·0–45·6	—	—	—	—	—	—	66·8	—	—
47·0	—	—	—	46·4	—	85·5	—	32·8	—
49·7–49·6	—	—	—	—	—	88·8	—	28·2	—
49·8–49·6	—	—	—	—	—	—	66·8	—	—
49·9–49·8	—	—	7·2	—	43·5	—	—	—	12·8
50·3	—	—	—	16·9	—	—	—	—	—
52·0	—	—	—	—	31·2	66·8	49·4	21·1	—
52·1–51·9	—	—	—	—	—	—	—	—	10·0
53·9–53·8	—	—	—	—	—	—	39·1	—	—
54·0	—	—	—	—	16·9	49·4	—	15·5	5·9

On plotting the percentage of maltose hydrolysed against the mean temperature of the experiment, these numbers give a series of optimum temperature curves from which the optimum temperature corresponding to each particular H^+ concentration of the medium may be read The curves are summarised for purposes of description in two figures (figs 2 and 3). In fig 2 are collected the curves corresponding to H^+ concentrations greater than the optimum reaction of fig 1; while in fig 3 are collected the curves corresponding to H^+ concentrations equal to, and less than, the optimum of fig 1.

Consider fig. 2. Here we have a series of curves of varying altitudes, which is what one would expect from the results already recorded in fig. 1; as the H^+ concentration of the medium is increased beyond the optimum value the enzyme is gradually rendered less efficient. And, in this respect, fig. 2 indicates, further, that the diminution in the activity is true for

practically all temperatures, although varying in amount from temperature to temperature, subject to the influence—to be explained presently—of the hydrogen ion concentration of the medium.

As to the influence, if any, of the H^+ concentration of the medium on the optimum temperature of the enzyme, fig. 2 clearly shows that for each H^+ concentration there exists a perfectly definite optimum temperature, also, the optimum temperature is seen to fall progressively as the H^+ concentration is increased beyond the optimum reaction of fig. 1 In fact, the locus



H^+ concentrations varying between 10^{-3} & $10^{-5.6}$

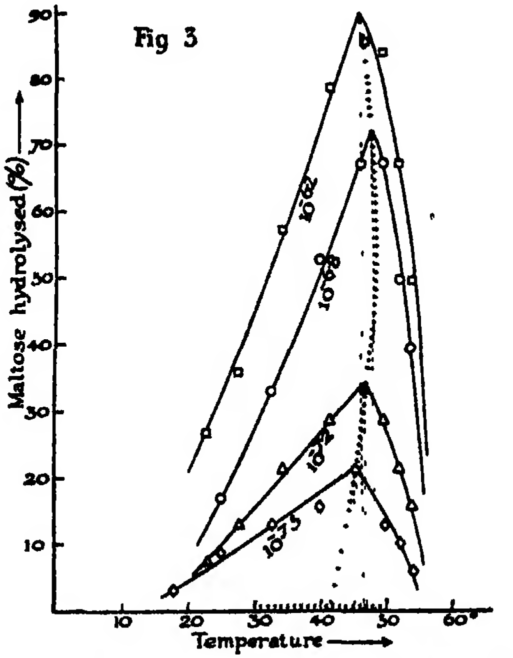
of the maxima of these curves is a straight line, the mathematical equation of which is approximately

$$y = 6\cdot56x - 200\cdot44.$$

The significance of this straight line is that it shows that the fall which occurs in the optimum temperature of the ferment as the H^+ concentration is increased—beyond its optimum value—is proportional to the fall in activity (or disablement) which the ferment undergoes at the physical optimum point.

Fig. 2 further shows that the temperature of destruction of the enzyme also depends on the hydrogen ion concentration of the medium; the greater

the acidity the less heat is the enzyme able to support before being entirely disabled Herein lies the explanation of the existence of the two lower curves of fig 2, in spite of the indications of fig 1, which shows that for the H^+ concentrations of $10^{-3.2}$ and $10^{-3.0}$ no activity on the part of the enzyme seems possible The reason is, that what was the optimum temperature for the natural reaction, +47°—at which temperature the results set forth in fig 1 were determined—is no longer the optimum temperature for the H^+ concentrations giving rise to the curves in question, but is instead a temperature of destruction



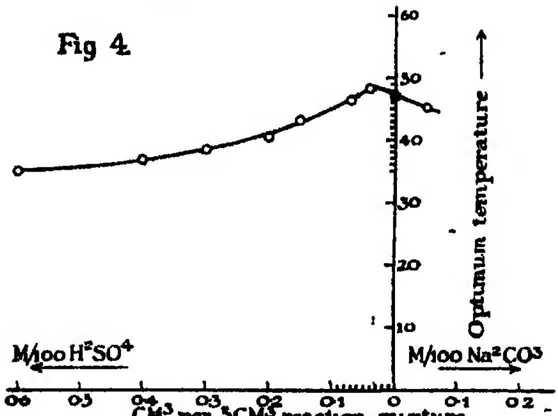
H^+ concentrations varying between $10^{-6.2}$ & $10^{-7.5}$

That the optimum temperature of the ferment diminishes as the H^+ concentration is increased beyond that of the chemical optimum may be stated otherwise thus: As the H^+ concentration is diminished from extreme values to values bordering on that of the chemical optimum, the optimum temperature steadily increases. An interesting question now arises. What would be the effect on the optimum temperature of diminishing the H^+ concentration beyond that of the chemical optimum? Would the optimum temperature under these circumstances continue to increase, in view of the fact that further diminution of the H^+ con-

centration of the medium must, in accordance with fig. 1, be attended by disablement of the enzyme? Fig. 3 answers these questions

Fig 3, as expected, also shows a series of curves of varying altitudes, and, as before, a perfectly definite optimum temperature is seen to characterise each hydrogen ion concentration studied But here an unlooked-for result is discerned For H^+ concentrations situated between the optimum and the natural reaction the optimum temperature rises still higher, to fall again as the natural reaction is overreached, the rise in the optimum temperature passes by an optimum value This is evident from the locus curve drawn through the maxima of the several optimum temperature curves figured in fig 3, and it is still better seen in fig 4, where a curve is plotted with the various optimum temperatures recorded in figs 2 and 3 as ordinates, and the corresponding amounts of acid and alkali present in the reaction mixture as abscissæ

Fig 4.



An optimum temperature of $+49°$ thus seems possible in a reaction mixture containing 0 032 cm^3 M/100 H_2SO_4 But it might be supposed that this is the optimum reaction of the ferment for that temperature, since, in accordance with the work of O'Sullivan and Tompson* on the enzyme *sucrase*, the optimum reaction of that ferment is known to depend on the temperature serving for its determination? This question it is proposed to investigate.

To illustrate in a striking way the essential fact established by the investigation, that the optimum temperature of the enzyme depends largely on the hydrogen ion concentration of the medium, it is only necessary to reproduce side by side on the same diagram the optimum temperature curve from fig. 3 corresponding to the natural reaction and that from fig 2

* 'Chem. Soc Journ,' vol 57, p. 859 (1890).

corresponding to the addition to the reaction mixture of 0·2 cm.^{3} M/100 H_2SO_4 per milligramme of enzyme This is done in fig 5

Fig 5.

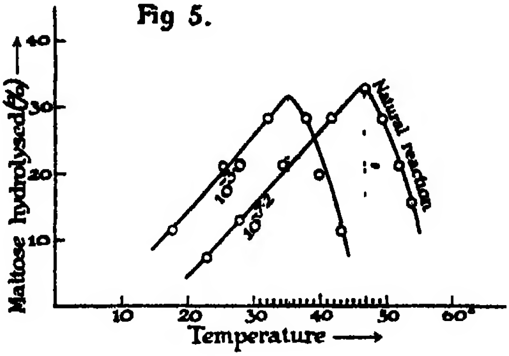


Fig 5 shows at a glance that by the simple process of increasing the hydrogen ion concentration of the medium in which the enzyme acts from $10^{-7 \cdot 2}$ to 10^{-3} it is possible to change the optimum temperature of the ferment from +47° to +35 5°,—*i e* through a range of 11·5° This result alone shows how important it is, when stating the optimum temperature of an enzyme, to point out at the same time the H^+ concentration of the medium serving to determine it Thus, the optimum temperature of the *maltase* in question, in an action of 16 hours' duration, may be expressed as $[35\ 5°]_{10^{-3 \cdot 0}}$ or $[47°]_{10^{-7 \cdot 2}}$

Fig 5 is further interesting from another aspect. The parallelism or similarity of the two curves is very striking; they are almost superposable Were they exactly so, and obviously that is only a question of sufficient experimental patience, it would mean that the activity of the enzyme is the same at corresponding temperatures over two equal although different ranges of temperature,—the one curve being in that case simply a translation in the plane of the paper of the other It is proposed to term this, the phenomenon of *corresponding states*. From a consideration of the locus lines of figs 2 and 3, it is evident that for H^+ concentrations such that the maxima of the resulting optimum temperature curves are situated at the same level on these lines, the enzyme should for the one and the other H^+ concentration be in "corresponding states."

The main conclusion of the present paper, in the insight which it affords into the influence of the H^+ concentration of the medium on the physical optimum of enzyme action, cannot perhaps be summarised to more advantage at present than by placing it in its appropriate place in a differential table briefly setting out our present knowledge of the relation of the physical and the chemical optima of enzyme action to the experimental conditions involved in determining them.

Optimum Temperature (Physical Optimum.)	*v*	Optimum H^+ Concentration (Chemical Optimum.)
i. Is independent of the concentration of the enzyme (Compton). *Maltase, salicinase.*		i. Ditto (Sörensen). *Sucrase.*
ii. Is independent of the concentration of the substrate (Compton). *Maltase, salicinase.*		ii. Is dependent on the concentration of the substrate (Van Slyke and Zacharias).† *Urease.*
iii. Is dependent on the H^+ concentration of the medium (Compton). *Maltase.*		iii. Is dependent on the temperature of the experiment (O'Sullivan and Tompson). *Sucrase.*
iv. Is dependent on the duration of the experiment serving to determine it (Bertrand and Compton).* *Amygdalinase, amygdalase.*		iv. Ditto (Sörensen). *Catalase, pepsin, sucrase.*
		v. Is dependent on the age of the enzymic preparation (Bertrand and Compton).‡ *Amygdalinase, amygdalase.*

* 'Comptes Rendus,' vol. 152, p. 1518 (1911).
† 'Journ. Biol. Chem.,' vol. 19, p. 205 (1914).
‡ 'Comptes Rendus,' vol. 159, p. 434 (1914).

The Life-Cycle of Cladocera, with Remarks on the Physiology of Growth and Reproduction in Crustacea

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1 *Experiments on* Daphnia pulex

In a paper on the life-cycle of *Moina rectirostris*, published in 1913 (5), it was shewn by the late Mr G H Grosvenor and myself that it was possible to inhibit entirely the production of the sexual forms by isolating the parthenogenetic parents soon after birth, and keeping them at a constant high temperature of 25–30° C It was proved that for a succession of eight generations the isolated parents at this temperature gave no males or ephippial females, while parents of the same generations kept crowded at a temperature of about 14° C or 5° C. gave about 50 per cent. males We were unable to determine how the effect of isolation and crowding of the parthenogenetic parents influenced the production of the sexual forms, but two alternative suggestions were made, either that in the crowded glasses the animals were unable to obtain sufficient nutriment and were partially starved, or else that some excretory matter accumulated in the crowded glasses which influenced the production of males and sexual females

In order to confirm the above results and to throw some light on the processes involved, breeding experiments have been carried on for some time with another species of Cladocera, the common *Daphnia pulex* Mr Robert Gurney very kindly gave me some dried mud from a pond which was known to contain the resting eggs of these animals, and, on placing the mud in a bowl of water, after some weeks some young Daphnia hatched out. One of these was kept until it had produced young, and the offspring of these young ones were used to start the first experimental generation

D pulex does not flourish on the food used for Moina, viz, manure infusion, but I had previously found that they could be cultivated with great ease if some green Alga, such as Protococcus, is added to the water in which they are kept In order to have a constant supply of the Alga, stock cultures were made in a nutrient medium of inorganic salts to which a small amount of organic material was added. The best medium for growing the Protococcus was found to be a certain dilution of the mixture recommended by Miquel for growing Diatoms, which Mr. H. G Thornton and myself have used for cultivating Euglena (8). By adding a pipette-full of the green growth to each glass in which the experimental animals are kept, it is possible

to ensure that there is always an excess of nourishment, because the culture consists only of the Alga and not of a mixed assemblage of bacteria, some of which may be useless as nourishment.

The scheme of the experiments is as follows —In each generation a certain number of the individuals are isolated soon after birth in separate glasses, some of these are placed in an incubator at 27° C, others are stood in a water tank with circulating water at 10–17° C Other individuals are kept crowded together in the same glass in numbers of 10–15, and of these crowded glasses some are again placed in the incubator and others in the circulating water at 10–17° C Thus in each generation we have individuals subjected to four different conditions —(1) Isolated at 27° C, (2) crowded at 27° C, (3) isolated at 10–17° C, (4) crowded at 10–17° C All are supplied with excess of Protococcus.

In Table I is given the result of breeding under these various conditions for eleven successive generations. This Table does not give the numbers of ephippial females which appeared among the parents, but it may be stated that ephippial or sexual females only appeared among the parents kept crowded at 10–17° C, which also gave a high percentage of males

It will be noted that in this Table, besides the numbers of male and female offspring produced in each generation, a column is devoted to the number of parents, whether isolated or crowded, which were used for breeding This factor, viz, the number of parents used, is one that must not be lost sight of, since, in order to prove that the production of males and sexual females is not simply a question of chance, it is clearly necessary to use a sufficient number of parents in each generation and under each condition to ensure that the effects of chance are ruled out. In as many cases as possible four broods were taken from each female It was not found that there was any tendency for later broods to produce more sexual forms than early broods

By consulting Table I it will be seen that neither in the isolated nor crowded individuals at 27° C did any sexual forms appear throughout the eleven generations. Adding the totals of the isolated and crowded at 27° C together we have, 90 + 150 = 240 parents gave 1188 + 643 = 1831 parthenogenetic females and no males.

Eighty-six individuals isolated at 10–17° C gave 1860 parthenogenetic females and 56 males, or about 3 per cent. males, while 420 individuals crowded at 10–17° C. gave 3564 parthenogenetic females and 256 males, or about 7 per cent. males.

These results are in agreement with our previous experiments with Moina, showing that the effect of isolation and high temperature is to suppress the production of the sexual forms (5)

Table I

Generation	Number of parents	Number of offspring: Female	Number of offspring: Male	Number of parents	Number of offspring: Female.	Number of offspring: Male
	Isolated at 27° C			Crowded at 27° C.		
1	5	35	0	20	47	0
2	13	43	0	20	65	0
3	13	80	0	—	—	0
4	4	14	0	10	20	0
5	1	22	0	16	28	0
6	11	103	0	20	171	0
7	12	331	0	16	147	0
8	9	253	0	24	105	0
9	7	120	0	16	20	0
10	6	75	0	8	40	0
11	9	112	0	—	—	—
Totals	90	1188	0	150	643	0
Generation	Isolated at 10–17° C			Crowded at 10–17° C		
1	5	129	0	20	91	7
2	8	204	0	33	1035	100
3	10	319	0	70	482	16
4	10	157	0	27	242	8
5	9	168	0	30	190	0
6	7	144	0	65	415	0
7	3	49	0	75	452	36
8	10	250	0	80	470	65
9	11	253	56	15	40	0
10	6	99	0	30	87	25
11	7	88	0	25	60	0
Totals	86	1860	56	420	3564	256

Since it was found that the individuals crowded at 27° C produced few young and did not flourish, the experiment was subsequently continued in a rather different way, only two kinds of conditions being employed, viz., isolation at 27° C and crowding at 10–17° C. In this second experiment as nearly as possible equal numbers of parents in each generation were used in the isolated and crowded condition Also a careful observation was kept to see how many of the parents used became ephippial or sexual. This experiment was made some time after the first with individuals that had been propagating by parthenogenesis, so that the first lot of parents used were about the 35th generation from the beginning of the cycle, *i.e.*, the original winter egg from which the first individual emerged.

The result which is given in Table II shows that during eight successive generations 117 isolated parents at 27° C. produced 2564 parthenogenetic females, no males, and in no case became ephippial, while 129 crowded parents became ephippial in 17 cases and produced 1147 parthenogenetic females and 26 males

Since nearly equal numbers of parents were used and more offspring were produced from the isolated parents than from the crowded, it is impossible to ascribe the production of sexual forms by the crowded individuals, and their entire absence in the case of the isolated parents, to chance

If we add together the results for the isolated at 27° C and for the crowded at 10–17° C. in the two experiments given in Tables I and II, we see that in nineteen generations 207 isolated parents at 27° C gave 3752 parthenogenetic females and no males, while 549 crowded parents at 10–17° C gave 4711 parthenogenetic females and 282 males, or about 6 per cent males It is also to be observed that, while no ephippial females appeared among the isolated parents at 27° C., about 10 per cent. of the crowded parents at 10–17° C became ephippial

Table II.

Generation	Isolated at 27° C				Crowded at 10–17° C			
	Number of parents	Parents becoming ephippial.	Number of offspring		Number of parents	Parents becoming ephippial	Number of offspring	
			Female	Male			Female	Male
35	8	0	132	0	10	2	63	10
36	16	0	334	0	20	1	117	0
37	19	0	414	0	20	0	222	0
38	16	0	350	0	10	1	111	0
39	18	0	397	0	20	4	116	0
40	9	0	158	0	10	0	264	0
41	16	0	644	0	10	2	59	1
42	20	0	140	0	29	7	195	15
Totals .	117	0	2564	0	129	17	1147	26

The general result of the above records is to show that in *D pulex*, as in *M rectirostris*, it is possible to inhibit entirely the appearance of males and sexual females by isolating the parents soon after birth and keeping them at a temperature of 27° C. But if we look into the numbers given for each generation in Tables I and II, we find that the converse of the above statement does not hold good, *i.e.*, it is not the case that crowding at 10–17° C. always results in the production of the sexual forms. Thus, to

take some instances in Generation 6, 65 crowded parents gave 415 parthenogenetic young and no males, in Generation 37, 20 crowded parents gave 222 parthenogenetic young and no sexual forms. It must be concluded from this that there is some factor involved in the production of the sexual forms other than external conditions, viz, an internal factor. That this internal factor is a regular rhythmical cycle, such as Weismann originally suggested (1), which runs on without any regard to external conditions, is obviously not true, but there is this very important element of truth in Weismann's view, namely, that a species such as *D. pulex* never produces as many sexual forms per cent as a species like *M. rectirostris*, and, as far as we know, no alteration of the external conditions would make it do so. The facts suggest that for each species of Cladoceran there is a maximal limit to the numbers in which sexual forms may be produced, and that this number cannot be readily increased but it can be decreased, or entirely abolished, by external conditions such as isolation and high temperature combined with abundant nutrition. If we suppose with Woltereck (3) that the production of parthenogenetic and sexual forms is due to the presence of two substances, a parthenogenetic substance and a sexual substance, then we should say that the relative amounts of these substances are fairly rigidly fixed for each species, and that, whereas the amount of the sexual substance cannot be easily increased, its operation can be indefinitely suspended by the action of external conditions. By this interpretation of the facts it is possible to retain the really important part of Weismann's theory, that the proportions in which the sexual forms are produced in each species is fixed in its upper limit in accordance with the adaptive necessities of the species, though we must maintain that these proportions can be altered by the suppression of the sexual forms through external conditions.

W. E. Agar (7) in a recent paper, after summarising the results of previous workers, comes to the conclusion that there is no such thing as an internal rhythm in Cladocera, and that the production of sexual or non-sexual forms is entirely controlled by the environment. I am in agreement with Agar in thinking that there is no hereditarily fixed rhythm, or that the production of the sexual forms is rigidly fixed on to particular generations or particular broods of these generations, but I find it impossible to believe that it is purely due to environment that a species like *M. rectirostris*, under any conditions, produces a far greater percentage of sexual forms than a species like *D. pulex*. In other words, there is an internal factor concerned in the production of the sexual forms, this factor varies in different species of Cladocera; its operation can be entirely suspended by external conditions so that no sexual forms are produced, but there is no experimental justification for the view

that the production of sexual forms can be provoked at will in any or every generation of a particular species by alterations in the environment.

It is important to consider whether the factor of crowding can ever operate in a state of nature in the same way as under cultural conditions. There can be no doubt that the way this factor exerts its effect is through the presence of some excretory material in minute quantities, because in our cultures of Daphnia, which were fed on nothing but Protococcus, it was possible to ensure that there was in all cases an excess of food, so that the crowding could never cause a shortage of food. Since the animals were cultivated in glasses containing about 100 c c water, and the presence of 10 individuals constituted the normal crowded condition, it is clear that the reaction must be a very delicate one, due to the presence of extremely minute proportions of the substance in question Now, in a state of nature, the small pools inhabited by many "polycyclic" species of Cladocera, *e g*, Moina, are often far more intensely crowded with individuals than under our cultural conditions But, quite apart from small pools, it is frequently to be observed that large ponds are often so thickly populated with species like *D magna* and *pulex* as to be coloured blood red, and I have met with cases where farmers have been afraid to water their horses at a pond on account of the extraordinary colour of the water I have also observed that even in lakes, certain areas of water may be intensely crowded with some species of Cladocera, and it appears to me probable that the factor of crowding may play its part in the production of the sexual forms even in the largest bodies of water. The interesting report of Dr Viktor Langhans on the Cladocera of the Hirschberg Lake in North Bohemia (4) shows that the various species of Cladocera inhabit for the most part quite localised areas of the lake, and, moreover, that the appearance of the sexual forms usually either coincides with or follows closely after the greatest activity in parthenogenetic reproduction, when crowding would be at its height.

2 *The Storage of Fat and Glycogen in its Relation to Growth and Reproduction in Cladocera.*

In the course of the breeding experiments described above, a contrast was noticeable between the individuals isolated at 27° C and those kept crowded at a lower temperature. It was observed, even on inspection with the naked eye, that the young or fully grown individuals isolated at 27° C. were always of a pale, translucent green colour, while those crowded at the lower temperature were generally bright reddish orange, or, at any rate, showed a good deal of this colour. On examining the two kinds of individuals under the

microscope it was found that the reddish orange individuals owed their colour to the abundance of coloured fat globules present round the gut and ovary and at the bases of the limbs, while the pale green individuals were either entirely devoid of any fat or else possessed a few globules in the neighbourhood of the ovary It was shown in a previous paper (6) that by placing living specimens of Cladocera, such as Moina or Daphnia, into a vessel of water in which a small quantity of neutral red is dissolved, it was possible to distinguish after a few hours certain bodies which took up the stain with great avidity These bodies which stain intensely *intra vitam* with neutral red are distributed in three chief situations—(1) as very small granules in the polygonal cells of the chitogenous ectoderm (fig 1) (in the case of ephippial females the chitogenous cells of the ephippium, which is formed of very thick chitin, contain much larger masses of glycogen, see fig 2),

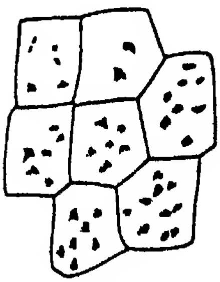

Fig 1

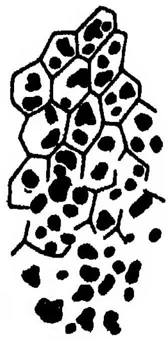

Fig 2

Fig. 1.—Chitinous areas on carapace, representing chitinogenous ectoderm cells, with small granules of glycogen stained with neutral red *intra vitam*.

Fig 2 —Chitinous areas on carapace in region of formation of ephippium, showing large lumps of glycogen, stained with neutral red

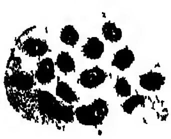

Fig 3.

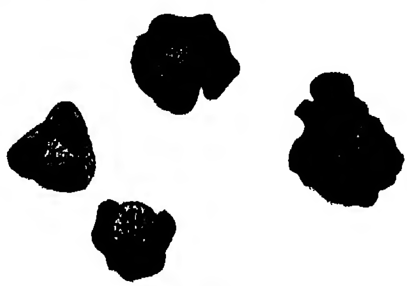

Fig 4.

Fig. 3 —A group of cells from the gut, showing small glycogen granules occupying each cell, stained with neutral red

Fig. 4.—Four large subcutaneous glycogen cells from base of limb, showing irregular masses of glycogen in periphery of cell, stained with neutral red.

(2) as small granules in the cells of the gut (fig. 3); and (3) as much larger, irregular-shaped masses in the connective tissue at the sides of the gut and at the bases of the limbs (figs. 4 and 5) There can be no doubt that these bodies are reserve material of the nature of glycogen, because the areas in which they occur are the same as those in which glycogen is known to occur in the higher Crustacea, and they exhibit the same appearance and staining reactions as the glycogen deposits in higher Crustacea The fact that the so-called glycogen deposits of Crustacea stain so intensely *intra vitam*, and also after fixation with neutral red, suggests that they are not pure glycogen, or, at any rate, not identical with the glycogen found in the liver of warm-blooded animals, because neutral red does not show any particular affinity for these latter deposits That they are largely composed of glycogen is shown, however, by their giving the iodine reaction both microchemically and, in the case of the higher Crustacea, after extraction with hot water in a test-tube reaction

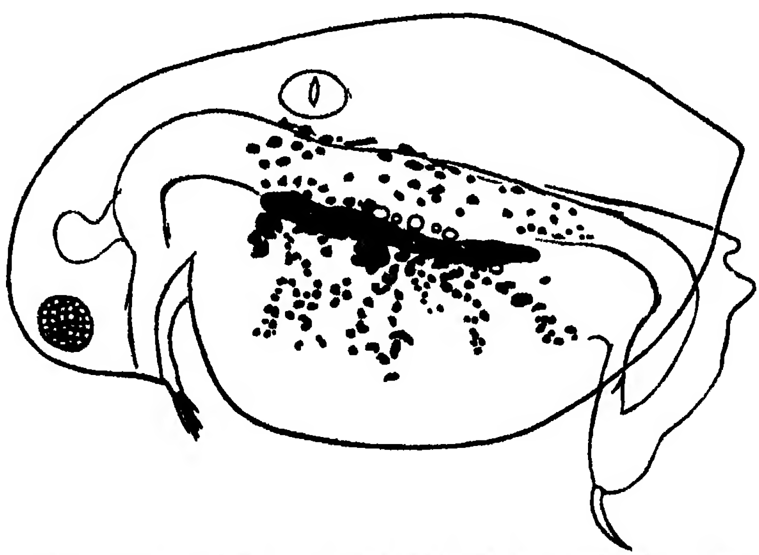

FIG. 5—Parthenogenetic female, isolated at 27° C, showing reserve material present almost entirely as subcutaneous glycogen, with a few fat globules in neighbourhood of ovary

It seems that the glycogen deposits in Crustacea consist of glycogen plus some nitrogenous material, probably a proteid derivative, which is responsible for the special affinity for neutral red. Leaving the exact chemical nature of these amylaceous deposits aside, it is to be observed that the pale green translucent individuals of *D. pulex* which have been kept isolated at 27° C. exhibit practically all their reserve substance in the form of this

glycogen material (fig 5), while the reddish-orange individuals which have been crowded at a lower temperature have the greater part of this glycogen replaced by orange globules of fat This does not apply to the small granules in the skin and gut, which are invariably present in all categories of individuals, but to the subdermal connective tissue masses round the gut and at the bases of the limbs. The connective tissue cells which store the reserve material have thus two alternatives they may store glycogen, as in the case of the pale translucent individuals, or they may store preponderantly fat, as in the case of the crowded individuals at low temperatures

By following the course of events occurring in the parthenogenetic females under the conditions of isolation at 27° C. and crowding at 10–17° C., it can be shown that the quite young individuals soon after birth in both cases have their reserve material distributed typically in the way shown in fig 6. There

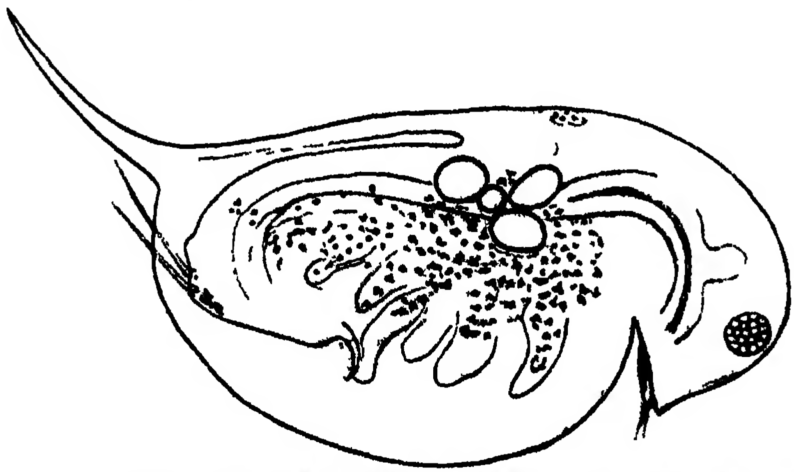

Fig 6.—A young parthenogenetic female, showing distribution of glycogen and the few large fat globules

are a few large globules of fat, represented by the dark circles, and a large supply of glycogen at the bases of the limbs. Now, as growth proceeds, the individuals kept isolated at 27° C retain their reserve material in the form of glycogen and do not develop fat in any quantity (fig 5), they grow and moult very rapidly and may reach maturity in three or four days. The individuals kept crowded at 10–17° C., on the other hand, tend to lose their glycogen deposits and to deposit large quantities of fat, and they grow and come to maturity much more slowly than the isolated individuals at 27° C. It is important to note here the coincidence of glycogen storage and rapid growth on the one hand, and of fat storage and retarded growth on the other.

Now the question arises, is it possible to connect this difference in behaviour

relative to reserve storage with the occurrence and non-occurrence of the sexual forms?

An examination of the condition of the sexual forms strongly suggests that an affirmative answer can be given to this question. The ephippial females are always bright orange in colour owing to the abundant presence of fat in all the subdermal tissue at the sides of the gut and at the bases of the limbs, while the ephippial ovary is characterised by the presence of closely packed globules of fat in the eggs and nurse cells. The appearance of an ephippial female with its abundant reserve fat and opaque ovary loaded with fat is shown in fig 7 In contrast with this the parthenogenetic female, even under the

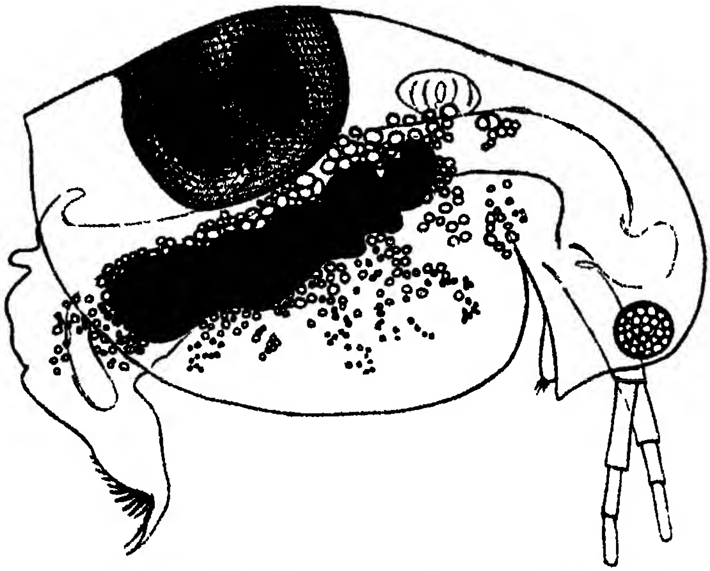

FIG 7.—An ephippial or sexual female with ephippium of two egg chambers and opaque ovary full of fat The reserve material present is in the form of very numerous fat globules of an orange red colour

crowded condition at low temperature, never exhibits so much fat in its reserve deposits, while the parthenogenetic ovary contains a very large quantity of amylaceous matter in addition to the comparatively sparse large fat globules in it. The adult males, as shown in fig 8, resemble the ephippial females in the abundance of fat present as reserve substance

Another point to be noted is that the ephippial females are inhibited in their growth and never attain to the same size as the parthenogenetic females kept isolated at 27° C, while the males are even more stunted.

We thus see that there is a remarkable coincidence between storage of glycogen and rapid growth on the one hand, and fat-storage and inhibition

of growth on the other, that the parthenogenetic females which are kept crowded at low temperatures tend to store fat in place of glycogen and to be retarded in growth, that this tendency reaches its maximum in the sexual forms, and that these sexual forms are produced only by the crowded parthenogenetic females which have a tendency to store fat and to be retarded in growth. The conclusion to be drawn from this series of facts is that the induced fat-storage and retarded growth of the parthenogenetic females crowded at low temperatures are the causal forerunners of the production of the sexual forms

If we regard the parthenogenetic mode of reproduction as being essentially

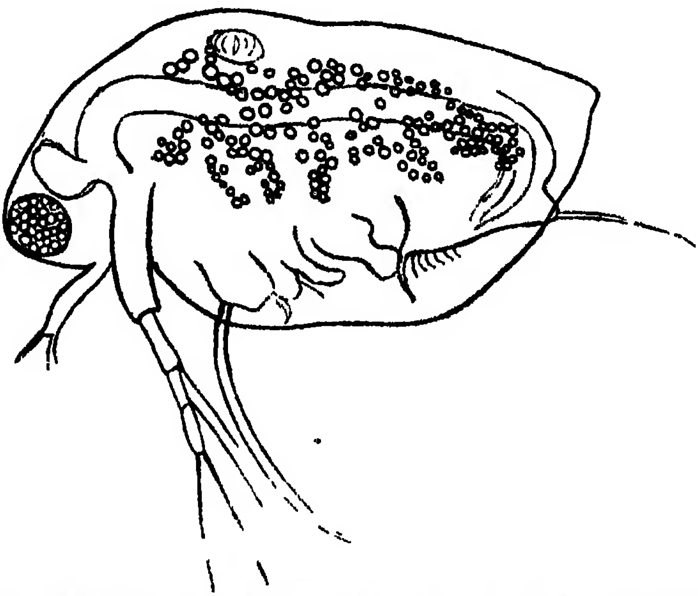

Fig 8 —Adult, fortnight old, male, showing reserve material present in the form of fat

a form of discontinuous growth or budding, we may observe that this is favoured by the conditions which also induce rapid growth in general, namely, preponderant storage of glycogen under the conditions of isolation and high temperature The production of the sexual forms, which grow slowly and reproduce with extreme tardiness, is accompanied by a preponderant storage of fat, under the conditions of crowding and low temperature.

It is claimed, therefore, that the manner in which external conditions determine the continuance of parthenogenesis or the production of sexual forms is as follows: The condition of isolation and high temperature favours the storage of glycogen as opposed to fat, and this storage of glycogen leads

to rapid growth and to continuous parthenogenetic reproduction, which is to be looked upon as a mode of growth by budding. The condition of crowding and low temperature, on the other hand, stimulates the storage of fat as opposed to glycogen, and this storage of fat tends to inhibit growth and to call forth the production of the sexual forms of male and female, which are pre-eminently characterised by abundance of fat-storage and retarded growth and reproduction Stated in a short and summary fashion, it is claimed that conditions which favour glycogen metabolism lead to rapid growth and parthenogenesis, while conditions which favour fat-metabolism lead to inhibition of growth and the production of sexual forms

The way in which the factor of crowding leads to fat-storage, inhibition of growth, and the production of sexual forms is still somewhat obscure But it is clear that the crowding does not act through partial starvation, because in all cases there was an excess of the food material present upon which the Daphnia were known to be feeding This was ensured by feeding the animals on a pure culture of green Protococcus, which constituted the sole food of the organisms. The only other way in which crowding can be conceived to exert an effect is by the accumulation of some excretory product in the water as the result of the presence of numerous individuals It is reasonable to suppose that this excretory matter might act in something the same way as phosphorus on a warm-blooded animal, namely, by stimulating the production of fat. All attempts at isolating or collecting this supposititious excretory matter have hitherto failed, and it would appear that it is easily destroyed, possibly by oxidation or bacterial action

3 *The Storage of Fat and Glycogen in its Relation to Growth and Reproduction in Decapod Crustacea*

We may now consider how far the theory of the connection between reserve-storage and growth and reproduction in the Cladocera harmonises with what we know of these processes in the higher Crustacea Ever since the writings of Claude Bernard and the more recent work of Vitzou (2), it has been known that the growth and moulting of the higher Crustacea is accompanied by a remarkable heaping up of glycogen in the liver and subdermal connective tissue. If we take sections through the liver of a crab, such as *Carcinus mænas*, which is about to cast its skin in the course of a day or two, it will be found, by staining the sections with iodine or neutral red, that the liver cells are crammed with small round granules of glycogen, to the exclusion of almost any other material (fig 9). At this period there is practically no fat and the protoplasmic content of the cells is small. Besides these storage cells of the liver, the ferment cells, with darker protoplasm and larger nuclei, will be

seen. In addition to the greatly increased glycogen deposits in the liver, cells containing large masses of glycogen are abundant in the subdermal connective tissue and in the tissue between the liver cells

If the liver of a crab in this condition is extracted for glycogen with hot potash solution, and the amount estimated as sugar by titration, the percentage of glycogen will be found to be very high, far higher than at any other time in the crab's life-history.

If now we take sections through the liver of a crab that has recently

FIG 9

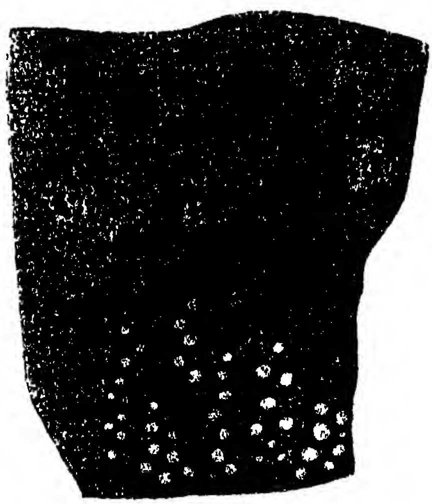

FIG 10

FIG 9—Section through portion of liver tube of Carcinus just about to moult. The storage cells are crammed with small round glycogen granules.

FIG 10—Section through portion of liver tube of ditto, some days after the moult. Reserve material is almost entirely absent from storage cells, which are full of protoplasm

completed its moult and has the shell soft and flexible, we shall find it in the condition shown in fig 10 The storage cells are now almost depleted of glycogen, and consist of protoplasm in which a few globules of fat, especially at the basal ends, are beginning to appear The subdermal glycogen will also be found to have very much diminished in quantity It is clear that the glycogen deposited in the liver and subdermal tissues just before the moult has been used up in the formation of the new skin and tissues during the rapid process of growth which follows the moult

If, finally, we take sections through the liver of a hard-shelled crab at a period intermediate between two moults, when growth is not proceeding, we

obtain the appearance shown in fig 11. Here the storage cells are seen to be filled with large and numerous fat globules, the only considerable stores of glycogen being found in the connective tissues outside the liver Three such connective tissue cells with glycogen are shown in fig 11 Quantitative estimations of the glycogen and fat in the liver and connective tissues under these various circumstances confirm the result obtained by histology, namely that during the moult there is abundance of glycogen (10 per cent.) and very little fat (3 per cent), immediately after the moult there is very little glycogen

FIG. 11—Section through ditto, about mid-way between two moults, when growth processes are in abeyance. The storage cells are crammed with fat globules Three connective-tissue glycogen cells are shown outside the liver

(0·1 per cent) or fat (5 per cent), and that between the moults there is abundance of fat (15 per cent) and a rather small amount of glycogen (1 5 per cent. (The figures given here are only rough average approximations, but they give a trustworthy idea of the relative proportions of fat and glycogen in the liver under the various conditions)

We conclude, therefore, that just as in the Cladocera, so in one of the higher Crustacea such as Carcinus, the period of active growth is accompanied by glycogen- as opposed to fat-metabolism, while the fat-storage in the liver

takes place in the intermediate periods when growth is in abeyance and the reproductive organs are maturing

In *Carcinus mænas* it was shown in a previous paper that the male and female differed in respect to their fat-metabolism (6) Thus in the female, maturing its ovaries, it was shown that the blood became flooded with lutein and fatty material to a much greater extent than the male, whose blood instead of becoming yellow with lutein is charged with the pink colouring matter tetronerythrin, but is not so heavily charged with fatty material as in the case of the female Coincidently with this it was pointed out that the female does not grow to the same size as the male, and this is again probably due to the fact that the preponderant fat-metabolism of the female for the nourishment of the eggs exerts a check on growth Finally it has been clearly shown that individuals of Carcinus infected with the parasite Sacculina do not increase in size at all after once the parasite has established its system of roots in the body of the crab, and this must certainly be ascribed to the fact that the Sacculina induces a most pronounced fatty habit in the liver of the crab, while the glycogenic function is permanently depressed. This has been completely proved by a series of quantitative estimations and histological examinations of the livers of infected crabs The condition of the sacculinised crabs, both physiological and morphological, is converted by the action of the parasite into that of mature females in the act of ripening their ovaries, and this, as we have seen, consists in a pronounced fatty habit and the inhibition of growth

The above considerations on the processes occurring in normal crabs and in those infected with Sacculina enable us to perceive that there is a marked agreement between these processes in the higher Crustacea and the conditions observed in the Cladocera In both cases active growth (in which parthenogenetic reproduction is included) is accompanied by storage and use of glycogen for building up the new tissues and skin, while inhibition of growth and sexual reproduction is accompanied by storage and use of fat for the nourishment of the sexual products This storage and use of fat is more pronounced in the case of the female than of the male, and it is clear that the fat-metabolism in the two sexes proceeds along different lines In the case of the female the fatty material developed and stored in the metabolic organs is merely transferred across to the ovary, while in the case of the male it appears that the fat storage is less pronounced and that the fat is not transferred as such to the testis in anything like the same quantity, but is broken down and used for other purposes

By thus bringing the processes of growth and reproduction in the Cladocera and in the higher Crustacea into agreement we obtain a certain

insight into the physiological basis of the antagonism between growth and sexual maturity which undoubtedly exists in the Crustacea, and the principle applies with modifications to organisms in general This antagonism is seen to be due to the necessity for the mature organism to produce a special kind of nutriment for the reproductive organs, so that there is a corresponding lack of the suitable reserve substances for the purposes of growth In the Crustacea and at some phase of the reproductive period in all organisms, the elaboration of fat for the supply of the ovary or accessory organs of reproduction is a marked feature of the metabolism in the mature female, and the diversion of reserve material in this form and for this purpose inhibits growth In the male it is less obvious in what special form the reserve material for the nourishment of the reproductive organs is prepared, but here, again, it is probable that fat plays an important part, though the manner of its utilisation is certainly different from the comparatively passive transference which occurs in the female The alteration in the metabolism thus brought about at sexual maturity, differing in its mode of operation in the male and female, we hold to be responsible for those morphological and physiological changes in the body which often accompany sexual maturity and are known as correlated secondary sexual characters

The view developed here as to the nature of sexual maturity and its antagonism to growth has an interesting bearing on the meaning of sex in general. Speaking broadly, the onset of the sexual mode of reproduction in organisms occurs under conditions when continued growth or asexual multiplication is hindered either by lack of appropriate food or accumulation of excretory matter or by some internal weakening of the assimilative capacity Under such conditions the organism responds by laying up reserve material for a special kind of resting reproductive cell instead of continuing to expend it in growth The sexual mode of reproduction is thus a means of lying dormant during conditions unfavourable to continued growth. The differentiation into male and female may be looked upon as an economy or division of labour by which the female reproductive cell stores up compactly a mass of reserve material to be used for the nourishment of the next generation, but thereby loses the power of division, while the male reproductive cell retains the kinetic energy for division but relies on the female cell to supply the material for development

Summary

1 By isolating the young Daphnia at birth and keeping them at 27° C. it has been possible to breed them for 19 generations without the appearance of males or ephippial females, 3752 parthenogenetic females having been produced

2. Parallel cultures to the above, when the parents are kept crowded to the number of 10 in a glass and at a temperature of 10–17° C, produced about 7 per cent males and 10 per cent ephippial females.

3 The crowding does not directly influence the supply of food, but appears to act by the accumulation of excretory matter in the glasses

4. The parthenogenetic females kept isolated at 27° C grow and reproduce more rapidly than those crowded at 10–17° C, and they store up reserve material almost exclusively in the form of glycogen, while the crowded parents at a lower temperature tend to store up fat instead of glycogen and are inhibited in their growth

5 The storage of fat as opposed to glycogen is especially characteristic of the males and ephippial females, hence it is judged that the fat-storage induced experimentally in the crowded parthenogenetic females at 10–17° C is causally connected with the production by them of the sexual forms.

6. We may conclude that the habit of glycogen-storage leads to rapid growth and parthenogenesis, which is a form of discontinuous growth, while the habit of fat-storage leads to inhibition of growth and sexual mode of reproduction

7 In the higher Crustacea the act of growth and moulting is accompanied by heaping up of glycogen in the liver storage-cells as opposed to fat, while in the periods between moults fat-storage preponderates

8. Preponderant fat-storage in the liver is characteristic of female crabs maturing their ovaries and of crabs infected by Sacculina, and in both these cases growth is inhibited.

9 We thus find that both in Cladocera and Decapoda growth on the one hand, and sexual maturity on the other, are accompanied by a different type of reserve storage, which is also distinct in the case of the male and female. This is the physiological fact at the root of the antagonism between growth and sex

10 Sexual reproduction is a reaction to conditions when continued growth is disadvantageous or impossible. Sexual differentiation is an economy or division of labour by which the female reproductive cell stores the material for development and thereby loses the power of division, while the male cell retains the power of division but relies on the female to supply the material for development

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Lepidostrobus kentuckiensis, *nomen nov*, *formerly* Lepidostrobus Fischeri, *Scott and Jeffrey* a *Correction*

By D H Scott, For Sec R S

(Received January 14, 1915)

In a paper by Prof Jeffrey and myself, published in the 'Philosophical Transactions,' last year,* we described a new species of *Lepidostrobus* from the Waverley Shale of Kentucky, under the name, *Lepidostrobus Fischeri* My friend, Prof R Zeiller of Paris, has now kindly pointed out to me that the specific name *Fischeri* is not admissible, another fossil cone having been described in 1890 by M B Renault, under the same name, *Lepidostrobus Fischeri* † I am sorry to have overlooked this reference, an oversight for which I am solely responsible

Our fossil must now receive a new name and it is unfortunate that it is no longer possible to record in the specific designation the name of the discoverer, Mr. Moritz Fischer The name I now propose for our cone is *Lepidostrobus kentuckiensis*, after the State in which the plant-bearing deposit occurs The diagnosis is briefly repeated below

* D. H Scott and E C Jeffrey, "On Fossil Plants, showing Structure, from the Base of the Waverley Shale of Kentucky," 'Phil Trans,' B, vol 205, pp 315-373 (1914)

† "Études sur le Terrain Houiller de Commentry —Flore Fossile, 2me partie," 'Bull Soc Industr. Min.,' 3e Série, IV, 2me Livr, p 526, Plate 61, fig. 3 (1890).

Lepidostrobus kentuckiensis, nomen nov

Lepidostrobus Fischeri, Scott and Jeffrey* (*non* Renault)

Cone large (4 cm in diameter to outer end of sporangia)

Sporophylls in about 35 vertical series.

Stele with a large "pith" of prosenchymatous cells, surrounded by a somewhat narrow ring of xylem with prominent angles

Leaf-traces with definite, confluent sheaths

Inner (or middle) cortex narrow, with an interwoven structure, but no gaps

Outer cortex very wide, prosenchymatous

Pedicels of sporophylls triangular in section, with a groove and median ridge on the upper surface, vascular bundle (rarely preserved) lying in soft tissue above the median ridge

Sporangia reaching 17 mm in length, with a palisade-wall and distal crest

Microspores in tetrahedral tetrads

Tetrads about 96μ, individual spores about $60 \times 48\mu$ in diameter, smooth.

From the base of the Waverley Shale, near Junction City, Boyle County, Kentucky, U S A

* 'Phil Trans,' B, vol 205, pp 354–363, Plate 29, phots 15–21, Plate 30, figs 20–23 (1890)

Investigations on Protozoa in Relation to the Factor Limiting Bacterial Activity in Soil.

By T. Goodey, M Sc, Protozoologist, Research Laboratory in Agricultural Zoology, University of Birmingham

(Communicated by Prof F W Gamble, F R.S Received December 3, 1914)

Introduction.

In the course of my work on soil protozoa particularly in relation to the question of the partial sterilisation of soil, I had occasion to work with some of the old stored soils kept at the Rothamsted Experimental Station, Harpenden, at which laboratory the work here recorded was commenced These soils are remarkable for the length of time they have been stored, 67 years being the longest period, and for the fact that in many cases the original samples put up in large bottles have remained untouched since the day on which they were bottled

Preliminary cultures of some of these soils in hay-infusion were begun in 1912 to ascertain the character of the protozoan fauna, if such still persisted in them. From these cultures it was found that in a mixed sample from Broadbalk, bottled in 1846 and containing about 3 per cent of water by weight, no protozoa were present, whilst in another mixed sample taken from six bottles of Barnfield soil, put up in 1870 and containing about 10 per cent of water by weight, amœbæ and flagellates but no ciliates were present.

Quantities of these two soils were taken and were submitted to partial sterilisation treatment in order to find out if the limiting factor usually eliminated by partial sterilisation was present in them. The results obtained by bacterial counts over a period of about 281 days showed that in the 1846 soil no factor limiting bacterial activity was present, whilst in the 1870 soil the limiting factor was present.*

As a result of this work I decided to use some of the 1846 soil for inoculation with different species of protozoa obtained from soil, in order to test if possible their power to act as the factor limiting bacterial activity The protozoa selected for culture and inoculation into separate samples of soil were the following:—*Colpoda cucullus*, *Col maupasii*, *Col steinii*, and *Vorticella microstoma* *Amœba* sps. ? and Flagellate sps ? were obtained by culture from the 1870 soil which, as already mentioned, had been found to contain

* This work was carried out in collaboration with Dr. H B. Hutchinson at Rothamsted.

the limiting factor, presumably the amœbæ and flagellates in it, according to Russell and Hutchinson's hypothesis.

Besides the above series of samples the set was made up to include a bottle of untreated soil, and one inoculated with a culture of bacteria representative of the bacterial flora added with the cultures of protozoa in the other samples, so as to serve as a check against them, and a bottle to receive 10 per cent of the 1870 soil, thus making nine bottles of soil in all

Another set of soils was experimented upon at the same time This consisted of seven bottles of fresh Hoosfield soil, partially sterilised first by toluene and then by heating to 65° C, so as to eliminate the limiting factor, and then inoculated again with cultures of protozoa obtained from the untreated soil. The series consisted of the following —Untreated, Toluened, Toluened + Untreated, Toluened + Ciliates, Toluened + Amœbæ, Toluened + Flagellates, Toluened + Bacteria. The bacteria used for the last-named inoculation were representative of the bacterial flora of the other cultures.

In each set of bottles the water content of the soil was finally brought to about 18 or 20 per cent by weight, this being about the water content at which many of Russell and Hutchinson's* soils have been maintained, and at which they have found the limiting factor to be active

I decided at the outset to make periodic bacterial counts by the gelatine-plate method in order to determine the numbers of bacteria in the soil as nearly as possible once a month and to carry on the experiments for a long period.

Methods.

A mixed sample of soil from six bottles of 1846 soil was taken and divided into nine lots of 400 grm in each.

Each lot of soil was put up in a quart bottle which had previously been sterilised and plugged with cotton wool.

In the case of the Hoosfield soil seven 400 grm. lots of slightly air-dried soil were taken after having been passed through a 3-mm. sieve. These were bottled in exactly the same way as the 1846 soil. The soil was first toluened by the addition of 2 per cent. of toluene, which was allowed to remain in the soil for two days, after which the soil was spread out on sheets of paper so as to allow the antiseptic to evaporate. Hay-infusion cultures of the toluened soil were made, and as it was found that flagellates developed in the cultures the bottles of soil were submitted to steam heat at a temperature of 63°–65° C. for three to four hours This operation was

* Russell and Hutchinson, 'Jour Agric Science,' vol 3, Part II (1909), and vol. 5, Part II (1913).

carried out in a steamer, the temperature of which was regulated by means of a thermostat Hay-infusion cultures were made after this second treatment, and it was then found that no flagellates cropped up

The cultures of protozoa used for the inoculation of the bottles of soil were obtained in the following manner. Hay-infusion cultures were made from fresh soil and from old cultures containing cysts which I had on hand By the use of fine capillary pipettes it was possible to isolate ciliates, which were then sub-cultured in hay-infusion I found it best to use hay-infusion already containing active bacteria for the sub-culture of isolated forms, the bacteria serving immediately as a source of food for the protozoa. Cultures of flagellates from the 1870 soil were obtained in the same manner, and for the culture of amœbæ from the 1846 soil I made use of cysts from pure cultures on agar plates which I had by me In this way pure cultures of the following protozoa were obtained for use with the 1846 soil —*Col cucullus, Col maupasii, Col steinii, Vort microstoma, Amœba* sp ?, and Flagellate sp ?

The protozoa for inoculation into the treated Hoosfield soil were obtained by isolation and sub-culture of forms cultivated in hay-infusion from the untreated Hoosfield soil, so that the forms added should represent as nearly as possible the fauna originally present in the soil The cultures of protozoa thus obtained were one of *Amœba* sp ?, one of Flagellates sp ?, and one of Ciliates, including *Col cucullus, Col steinii*, and *Col maupasii* The small Ciliate *Balantiophorus minutus* or *elongatus* also occurred in the cultures made from the untreated soil, but as I was unable to obtain this free from flagellates, the culture of ciliates did not include this form

In order to obtain mass cultures of the protozoa in sufficient quantity to serve for inoculation into the soil the following method was employed 80-grm. lots of washed and sterilised sand were put into large sterile petri dishes or glass cylinders and covered with hay-infusion, which was then infected with a pure culture of protozoa, and the latter were allowed to multiply and populate the culture.

In this way a large quantity of each kind of protozoa was obtained for the inoculation of the soil This process was carried out by spreading the soil on sheets of sterilised brown paper and then mixing the sand-hay-infusion culture of protozoa into it by means of a sterilised spoon, the whole of the soil, sand, and hay-infusion becoming thoroughly well mixed together and thus ensuring an even distribution of the protozoa throughout the soil In the case of the 1846 soil the inoculation was carried out in a glass-house which had been steamed down in order to allay dust and thus minimise the chance of infection. The soils were left exposed in this house for some days

in order to allow the bulk of the water to evaporate off, and a heating lamp was put into the house in order to accelerate slightly the evaporation

The reason for thus driving off the bulk of the water from the soil was that I desired to bring about in the soil all the conditions possible for aiding the excystation of the added encysted protozoa, for I had found in experimenting with cysts that if they were slightly air-dried and then moistened, excystation was more rapid than where no slight drying had been allowed

The water-contents of the inoculated soils, when bottled again at the end of these few days of air-drying, were as follows —Untreated, 6 2 per cent, Untreated + Bacteria,* 2 7 per cent, U + *Col cucullus*, 7 4 per cent, U + *Col. steinii*, 7·22 per cent., U + *Col. maupasii*, 6 9 per cent, U + *Vort. microstoma*, 6 8 per cent, U + *Amœbæ*, 7 5 per cent, U + Flagellates, 7 5 per cent. U. + 1870, 6 5 per cent.

Sterile distilled water was next added to all the soils, in sufficient quantity to bring up the water-content of each to 18 per cent

In the case of the seven bottles of Hoosfield soil, the samples were inoculated with protozoa from the sand-hay-infusion cultures in exactly the same way as described above These lots were spread out on sheets of sterile brown paper and left in the drying room for five hours at a temperature of about 20° or 22° C. in order to drive off the bulk of the added water and bring about conditions favourable to the excystation of protozoa after re-moistening The water-content of the various soils after drying was as follows —Untreated (U), 7 4 per cent., Toluened and heated (T), 5·9 per cent., T + Untreated (T + U), 5 8 per cent., T. + Ciliates (T + C), 3 1 per cent., T + *Amœba* (T. + Am), 2 7 per cent., T. + Flagellates (T. + Fl), 3 per cent, T + Bacteria (T + B), 1 6 per cent. Sterile distilled water was then added, as in the case of the 1846 set, in order to bring up the water-content of each lot to 18 per cent. by weight.

Both sets of bottles were then left in a small warmed glass-house, the temperature of which varied between 45° and 55° F. Later on they were taken from the glass-house and kept in the laboratory in a room at about 12–15° C. At various intervals during the course of the work the water-content of each soil has been determined in order to estimate the loss of water by gradual evaporation, and at these times the loss from each has been made good by the addition of sufficient sterile distilled water to bring up the water-content to 18 or 20 per cent. by weight

In attempting to estimate the numbers of protozoa present in the soils the following methods have been employed —

* This lot was treated in the same manner as the inoculated Hoosfield soil described further on.

A dilution method was first used which Dr. H. B. Hutchinson had devised and which he and I had used in 1910 in the course of some joint work. Ten grm. of soil are shaken up for four minutes with 100 c c of 1-per-cent hay-infusion, either sterile or containing an active growth of soil bacteria. By means of sterile 1 c c pipettes varying quantities of this soil suspension are taken out and placed in sterile tubes, three tubes of each dilution being put up In the case of the smallest quantities of soil suspension more hay-infusion is added in order to give a sufficient quantity of liquid for purposes of manipulation. The scheme of dilution is as follows :—

		grm of soil
10	c c original soil suspension	= 1
5	" "	= 0 5
2	" "	= 0 2
1	" "	= 0 1
0 5	" "	= 0 05
0 2	" "	= 0 02
0 1	" "	= 0 01
0 5	c.c mixture of 1 c c original + 9 c c hay-infusion	= 0 005
0 2	" " " "	= 0 002
0 1	" " " "	= 0 001

The cultures thus obtained are allowed to incubate for about a week and microscopical examination is made of the surface layers by taking out drops on a sterile platinum loop and examining them on glass slides If protozoa occur in the cultures of any particular dilution, then one infers that they are present in the soil in numbers equal to the factor required to raise the particular dilution to 1 grm. Thus if they occur in all three cultures of 0 001 grm, then there are at least 1000 protozoa per gramme of soil The method possesses the advantage that one deals with a comparatively large quantity of soil, viz 10 grm, and should thus be able to overcome the difficulties of any irregular distribution of protozoa in the soil itself, provided a good suspension is made However, in working with it I have obtained most irregular results, which I have not been able to explain, and for this reason I have practically given up using it in favour of an agar-plate method.

Before leaving the hay-infusion method, however, I may add that I carried out a series of experiments in order to ascertain if the violent agitation of the soil and liquid, in making the suspension by shaking for four minutes, had any injurious effect on living protozoa I found, when soil was added to a hay-infusion culture containing innumerable active ciliates and apparently no encysting forms and the mixture was shaken violently for four minutes, that on making a series of dilution cultures from this suspension protozoa

cropped up in abundance throughout, thus showing that they had suffered no damage

The agar-plate method which I have used is as follows —Sterile petri dishes are poured with nutrient bouillon agar of about 0 5 to 1 per cent in strength, and, when cool, the surface of the agar is inoculated with a weighed quantity of the soil the number of protozoa in which it is desired to ascertain. Three plates, as a rule, are inoculated with each weight of soil and the following are the weights of soil which have been used—1, 0 5, 0·2, 0 1, 0·05, 0 02, 0 01, 0 005, 0 002, 0 001, 0 0005, 0 0002, 0·0001 grm The plates are allowed to incubate for a few days and then the surface of each is examined under the microscope for the presence of protozoa The method entails the use of a sensitive balance and is limited by the difficulty of manipulating such small quantities of soil as are produced in weighing in the region of 0 0001 grm However, the results which I have obtained with it are fairly consistent and are more trustworthy than those of the hay-infusion method, I think

It was my hope at the beginning of the experiment to obtain evidence, by means of the counts of protozoa, concerning their activity and multiplication if such were proceeding

Counts of protozoa were therefore made at the beginning and towards the ends of the experiments. The hay-infusion method was used in the first counts and the agar-plate method for the later ones As I have pointed out, the latter method gives higher counts and more trustworthy results, and one cannot, therefore, strictly compare the evidence afforded by the two methods.

For this reason I have not found it possible to obtain sound evidence as to whether the protozoa have multiplied since being added to the soil See footnote, however, on p 454

The Bacterial Counts

The results of the periodical determination of the numbers of bacteria by the gelatine-plate method are tabulated below and the curves obtained by plotting these results are shown in figs 1, 2, 3, 4, and 5.

In order to simplify matters I have arranged certain curves together, for the whole nine curves when plotted all together present a confusing array and do not lend themselves to easy elucidation.

Fig 1 shows the curves obtained from the untreated, bacteria, and *V microstoma* inoculated soils.

The most noteworthy feature is the extraordinarily high bacterial count in the Bacteria soil at 32 days and the subsequent drop in the numbers of bacteria to a level below that of the untreated soil. This low bacterial

Bacteria in millions per gramme

	At beginning	After 32 days	After 63 days	After 92 days	After 124 days	After 153 days	After 181 days	After 282 days
Untreated	6·6	891	291	307	280	203	261	118
Bacteria	15	1504	472	440	259	98	102	59
Col cucullus	235	464	386	312	815	174	266	159
Col. steinii	218	379	314	271	289	165	324	142
Col maupasii	253	501	262	818	260	191	264	116
Vort microstoma	186	453	216	201	156	65	119	84
Amœba	178	599	412	274	409	193	242	178
Flagellates	82	374	827	416	319	127	188	132
U +'70	8 5	204	154	169	128	116	159	103

	After 284 days	After 324 days	After 360 days	After 383 days	After 419 days	After 486 days	After 519 days
Untreated	50	55	lost	115	76	87	57
Bacteria	lost	27	40	36	20	45	33
Col cucullus	96	102	143	104	95	134	70
Col steinii	77	188	112	224	133	138	130
Col maupasii	75	116	126	131	114	133	91
Vort microstoma	35	86	39	95	67	91	58
Amœba	160	185	129	242	160	168	118
Flagellates	54	86	98	120	75	124	128
U +'70	84	83	86	111	76	93	68

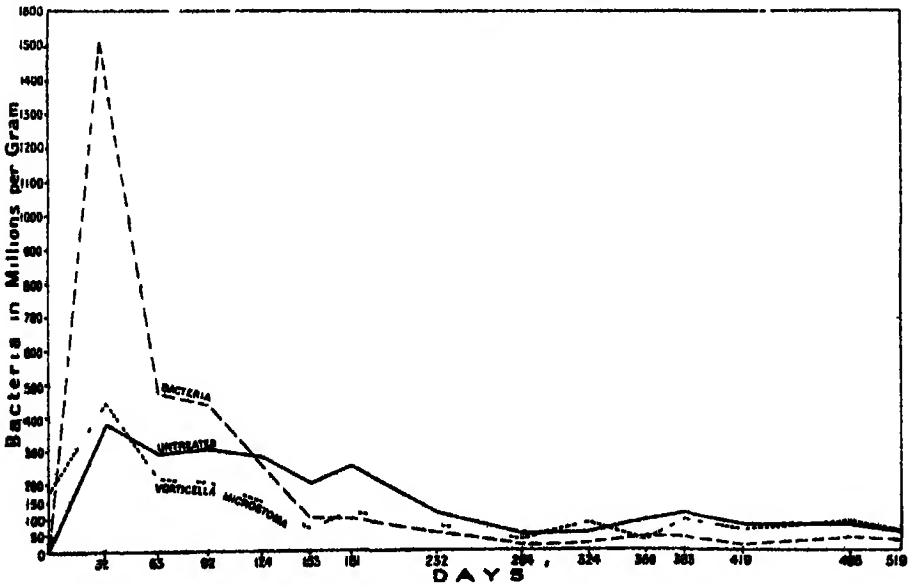

Fig. 1.

content was maintained over a very long period—366 days—and is perhaps the most surprising and unexpected result of the whole investigation.

The *V microstoma* curve is also very interesting and shows the influence of some factor which had become operative by the end of 63 days and which subsequently kept the numbers of bacteria in check, though only at about the same level as the untreated soil

The untreated curve also shows that the bacteria have gradually decreased in numbers after reaching and maintaining a high level for 181 days. These results are very interesting when considered in relation to the number of protozoa in the soils

In the untreated 1846 soil no protozoa are present, so that the gradual decrease in the number of bacteria cannot be due to the activity of the protozoa The *V microstoma* soil, however, contained, a few weeks after inoculation, about 300 vorticellæ per gramme By December, 1913, however, all the vorticellæ had died out, for they failed to appear in cultures of the soil made at that time and on all occasions since * Flagellates and amœbæ are present in this soil, probably due to infection of the mass culture or during the initial air-drying of the inoculated soils, to the extent of 1000 flagellates and 100–200 amœbæ per gramme

It is probable that these lead an active trophic existence in the soil and so might be considered responsible for the limiting action on the bacteria This can scarcely be the case, however, when we consider this soil in relation to the bacteria-inoculated soil In the latter there are flagellates present to the extent of about 100 per gramme If now the limiting factor in this soil is considered as due to the action of these flagellates, we should expect to find not so great a decrease in the bacterial numbers as in the vorticella soil, where the flagellates are more than ten times as numerous, and where there are, in addition, 100–200 amœbæ per gramme The reverse of this is the ascertained result, and clearly negatives the idea that the flagellates and amœbæ are responsible for the limiting action on the bacteria.

Another point of interest is that the two curves for the untreated and

* This dying out of the *Vorticella microstoma* is very interesting At first I thought its failure to appear might be due to an unsuitable culture medium. I, therefore, tried to obtain it again, taking care of the reaction of the hay-infusion, but with no better success It also failed to appear on a nutrient bouillon agar, favourable to the growth of all the other protozoa under consideration I afterwards remembered that in some earlier experiments I had failed to obtain Vorticella from a soil which had been kept in the laboratory for some months and from which I had obtained a very fine culture of the organism when the soil was fresh At another time too I failed to get the excystation of *V microstoma* from cysts which I had obtained in a hay-infusion culture and which had been stored for a few months From this evidence it would appear that *V. microstoma* in its encysted condition does not retain its vitality for more than a few months, and the dying out in my soil is easily accounted for on this supposition.

V microstoma soils are, during the greater part of their course, so closely akin that within the limits of experimental error they may be considered identical In one of them no protozoa are present, whereas in the other amœbæ and flagellates are present If the latter are the limiting factor they have failed to reduce the numbers of bacteria below the level of the untreated soil containing no protozoa.

Fig 2 shows the curves for the three samples of soil inoculated with the

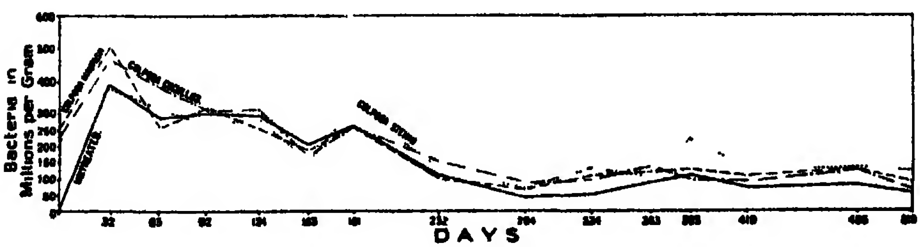

Fig 2

three different species of Colpoda, viz, *Col cucullus*, *Col steinii*, and *Col maupasii*, together with the curve of the untreated soil

There is a marked similarity between all four curves, all of them are of the same type and show no very pronounced differences On the whole, the bacterial content of the three inoculated soils has remained higher than that of the untreated soil, in spite of the fact that each contained many hundreds of protozoa per gramme They thus fail to indicate any action by the protozoa of a limiting character on the bacterial population of the soil

In the *Col cucullus* soil there are, roughly, about 750 *Col cucullus* and 1000 flagellates per gramme, the latter only being found in the later determinations by the agar-plate method. The *Col. steinii* soil contains about 100 *Col steinii* and 1000 flagellates per gramme, whilst the *Col. maupasii* soil contains about 1000 *Col. maupasii*, 200 amœbæ, and 100 flagellates per gramme.*

One would have expected that had the protozoa been capable of acting as a check on the growth of bacteria in the soil, they would have brought their numbers to a level well below that of the bacterial content of the untreated soil. Instead of this, however, we find after 519 days all three soils showing a higher bacterial content than the untreated soil.

* The numbers given for the protozoal counts are those obtained in the last determination.

The amœbæ which occur now in these soils are due most probably to infection either of the soil samples during the initial air-drying or of the mass cultures

This is good evidence, I think, that the protozoa have not functioned as the limiting factor

In the case of the soils inoculated with cultures of amœbæ and flagellates obtained from 1870 soil, the curves for the bacterial counts of which are shown in fig 3, the general inference to be drawn is that after a period of

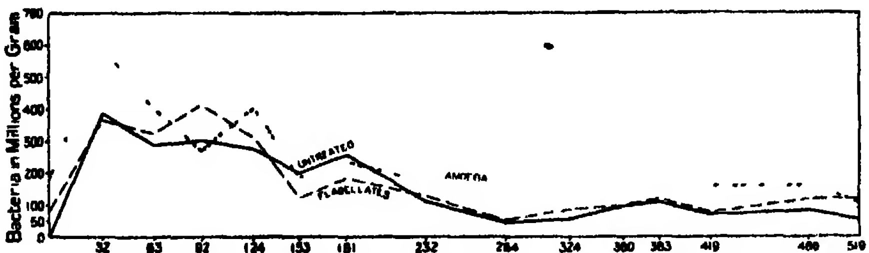

Fig 3

18 months in which to act, the protozoa have not exerted a limiting action on the bacteria in their respective soils

At 519 days the bacterial content of both inoculated soils is well above that of the untreated soil.

The amœbæ in the sample of soil specially inoculated with them are present to the extent of 10,000 per gramme, whilst in the flagellate-inoculated soil there are 10,000 flagellates and about 2000 amœbæ per gramme These results are very interesting, for they indicate that even when protozoa are present in the soil in such large numbers and under conditions favourable to active existence they do not exert a depressing effect on the bacteria

The amœba curve is especially significant, for it shows that even in the presence of 10,000 amœbæ per gramme of soil the bacteria can maintain a higher level in numbers than in the original soil containing no protozoa.

The curve for the flagellate-inoculated soil does not call for much comment. It is practically identical with that for the untreated soil during a great part of its length, but on the whole is at a higher level and indicates that the 10,000 flagellates and 2000 amœbæ per gramme are not capable of bringing the bacterial content down below the level of the untreated soil.

Fig. 4 shows the curves for the untreated soil and for the sample to which 10 per cent. of 1870 soil was added. At the end of 519 days the untreated soil is at a lower level than the U.+1870, though from 232 days onwards the two curves are very similar, and from 360 days to the end of the experiment may be considered as identical. There are about 2000 amœbæ and 2000 flagellates per gramme in the mixed soil, and the curves show that these have not been able to bring down the bacteria to a level below that of the

untreated soil (see footnote, p 454) The Untreated + 1870 curve is, on the whole, very even, showing no marked fluctuations up or down, and it is very

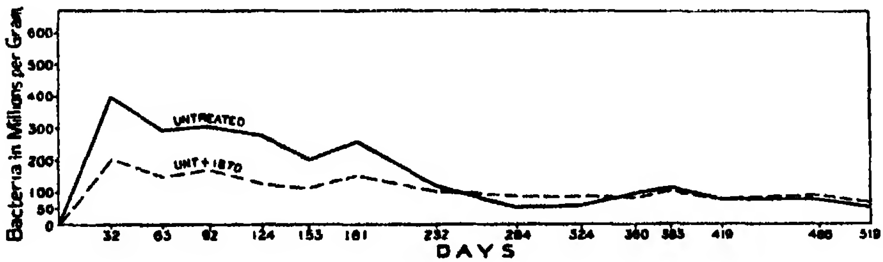

Fig 4

interesting to note that during the first 232 days the bacterial counts are below the counts for the untreated soil This seems to indicate that some factor was introduced with the 1870 soil which, for a time, checked the rapid growth of bacteria and prevented their increasing to the numbers attained by the bacteria in the untreated soil It is conceivable that this was owing to the action of the protozoa added with the 1870 soil, but the fact that after 232 days the bacterial numbers for the untreated soil came down below the level of the mixed sample, and that the two curves during the last 160 days are practically identical lends no support to this idea.

Whatever the influence was which during the first 232 days checked the growth of the bacteria in the mixed soil, I am inclined to regard it as connected with some other property of the 1870 soil than the presence of protozoa in it To be more explicit The 1870 soil was bottled in a comparatively moist condition and received no drying about 1881 as did the 1846 soil along with many others. This drying seems to have effected a very important change in the 1846 soil and, taken in conjunction with the prolonged period of storage has produced in it a condition comparable with partial sterilisation At any rate, the 1846 soil along with other dried and stored soils which I have examined gave very high bacterial counts when moistened, whereas the 1870 and other soils which have been stored in almost the same condition as they were in when taken from the field give low bacterial counts and indicate the presence of the limiting factor. The following experiments illustrate my point.

Bacterial Counts in other Samples of Old Stored Soils.

Three soils were taken for this piece of work, two of them from bottles of Broadbalk soil stored since 1856 and 1865, and one from a bottle of Geescroft

soil stored since 1865 The two from Broadbalk were dry when taken from their original bottles, whilst the Geescroft sample was in a comparatively moist condition

The water-contents of these soils were not determined immediately after taking them from their respective bottles, because it was not my intention at that time to use them for bacterial counts but merely to ascertain the character of the protozoan fauna. As far as I could judge, I should say that the Geescroft soil contained about 10 per cent of water, whilst the Broadbalk samples were of about the same degree of dryness and contained about 2 per cent or 3 per cent of moisture It was evident from the appearance of the soils that the Broadbalk soils had been taken out and dried along with many other soils in 1881, whereas the Geescroft soil had been left untouched and closely resembled the Barnfield 1870 soil, containing about 10 per cent of water, which I had used in earlier work

I found on examining these soils culturally that the Broadbalk 1856 contained no protozoa, the Broadbalk 1865 contained amœbæ and flagellates and the Geescroft 1865 also contained amœbæ and flagellates

A weighed quantity of each of these soils was taken and after making initial counts to determine the bacterial content they were all moistened to 20 per cent. water-content. After a period of 148 days the soils were remoistened to bring up the water-content to 20 per cent again, owing to the gradual loss of moisture by evaporation

Bacterial counts by the gelatine-plate method were made at different intervals and the results are set out in the table below

Bacteria in Millions per Gramme.

	At beginning	After 68 days	After 102 days	After 130 days	After 198 days	After 281 days.
Bd 1856	4	136	169	73	200	158
Bd 1865	4	66	93	55	120	124
G 1865	2 5	11 4	5	10·7	18·6	9

The curves obtained on plotting these results are shown in fig. 5.

The most noteworthy feature of these curves is the high bacterial content of the Broadbalk soils and the low bacterial content of the Geescroft soil. The former have all the appearance of curves of partially sterilised soils, whilst the latter presents the usual appearance of an untreated poor soil, containing a limiting factor.

The drop at 130 days in the two Broadbalk soils may be accounted for by

the loss of moisture, and the subsequent rise in the bacterial content may likewise be attributed to the more favourable conditions occasioned by remoistening the soils.

Considered in relation to their protozoan fauna, these results are very instructive In the Broadbalk 1856 there are no protozoa In the Broad-

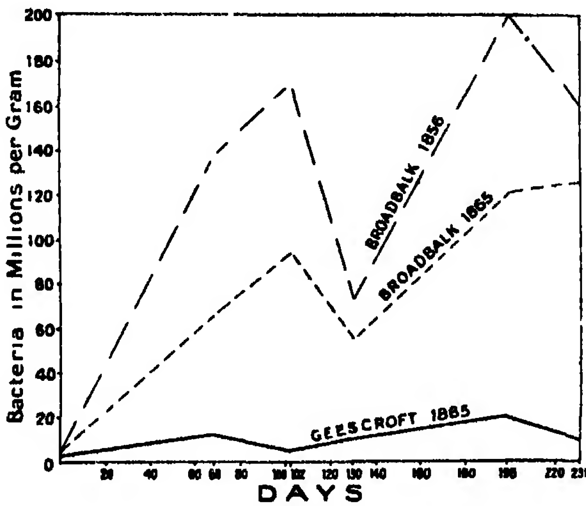

Fig 5

balk 1865 there is a rich protozoan fauna to the extent of about 5000 amœbæ and 5000 flagellates per gramme. In the Geescroft 1865 there are about 500 amœbæ and 500 flagellates per gramme Thus the curve for the Broadbalk 1865 shows that in spite of the presence of this large number of protozoa, the bacteria can maintain a high level in numbers even after 231 days during which the protozoa should have been reducing them.

It may be suggested that the bacterial counts for Broadbalk 1856 are higher than those for Broadbalk 1865 because in the former there are no protozoa present to check the growth of the bacteria I would point out, however, that the two Broadbalk curves are practically of the same order as compared with the Geescroft curve One would have thought that in the presence of such a large number of protozoa as in the Broadbalk 1865, had these been capable of functioning as the limiting factor, they would have checked considerably the multiplication of the bacteria and brought them down to somewhere near the level of the bacterial content of the Geescroft soil

My point is to show that the drying to which the Broadbalk soils were submitted has brought about a change in them strictly comparable with the change usually produced by partial sterilisation, and at the same time has

produced this change in one of them without killing off the amœbæ and the flagellates.

The Geescroft soil remained undried and has a much scantier protozoan fauna than the Broadbalk 1865 soil, yet the curve for the bacterial counts in this soil would be interpreted as showing the presence of the usual limiting factor.

I have no evidence on which to base a suggestion as to what the real character of the change is which has been produced in the Broadbalk soils by drying. I do suggest, however, that it has an intimate relation to the high bacterial counts which I have obtained on remoistening the soils I am quite prepared to admit that the merely negative evidence furnished by the above results does not help forward very much the final solution of this elusive problem, but at the same time I think that it is useful It points to the fact that much more information is required than is at present available on the changes brought about in soil by rapid air-drying or by drying at temperatures sufficiently low to avoid the killing of protozoa in the soil

Russell and Hutchinson give details of several experiments on this particular line of investigation in their second paper (p 166), but there is room for still more research on these points.

Hoosfield Inoculated Soil Bacterial Counts

The results of the bacterial counts for this set of soil samples are tabulated below, and the curves obtained by plotting these are shown in figs 6, 7, and 8 As in the case of the 1846 set of soils I have arranged certain curves together for the sake of simplifying matters.

It is necessary to point out at the outset that in attempting to interpret these results there are two standards of comparison, viz, the curve for the untreated soil and that for the toluened soil. For this reason I have introduced each of these curves into all three graphs.

Fig. 6 shows the curves for the Untreated, Toluened, T + Ciliates, T. + Amœbæ, and T + Flagellates It will be seen that the untreated soil exhibits a normal low bacterial content, the limiting factor is here exerting its full influence. Compared with the untreated, the curve for the toluened soil shows that the usual partial sterilisation effect has been obtained, the bacterial numbers rising to and maintaining a level at about 50,000,000 or 60,000,000 bacteria per gramme. Examining now the curves of the three inoculated soils represented in this graph and comparing them especially with the curve for the toluened soil, we find that after the lapse of 487 days the bacterial contents are higher than that of the toluened soil. Leaving

Bacteria in millions per gramme

	At beginning	After 32 days	After 60 days	After 93 days	After 125 days	After 151 days	After 173 days	After 208 days
Untreated (U.)	14 4	10 8	13	11 4	9	12	13 5	8
Toluened (T.)	9 2	73	60	61	48	40	53	49
T.+U.	11 3	40	61	43	70	19	39	45
T.+Ciliates	4 5	371	202	296	181	56	70	64
T.+Amœbæ	8	285	185	141	188	74	72	90
T.+Flagellates	27	247	214	227	368	196	108	104
T.+Bacteria	2 3	500	341	311	296	181	196	151

	After 259 days	After 301 days	After 337 days	After 350 days	After 386 days	After 454 days	After 487 days
Untreated (U.)	6	13	lost	8	13	12 6	12
Toluened (T.)	42	55	70	67	43	51	56
T.+U.	23	39	44	51	33	33	48
T.+Ciliates	50	57	65	59	57	57	73
T.+Amœbæ	57	65	68	62	50	41	57
T.+Flagellates	77	104	92	94	81	35	113
T.+Bacteria	116	151	85	105	138	115	150

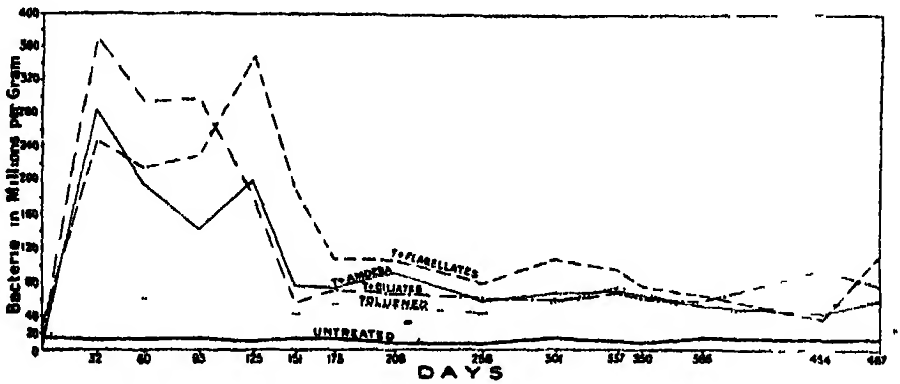

FIG. 6

out of account for the moment the high bacterial counts during the first 125 days and the drops at about 150 or 170 days in all three cases, we may say that the protozoa added to the toluened soil in each sample have not reduced the bacteria to a level lower than that of the toluened soil alone. Judged in relation to the protozoa in these soils this is a very instructive result. The T.+Amœbæ contained at the end of the experiment 10,000 amœbæ, perhaps more, and about 5000 flagellates per gramme. The T.+Flagellates contained 10,000 flagellates, perhaps more, and about 1000 amœbæ per gramme, whilst the T.+Ciliates contained about 3000 each of *Col. steinii* and *Col maupasii*, about 500 *Col. cucullus*, and about 1000

flagellates per gramme These are large numbers of protozoa, and it is probable that the amœbæ and flagellates have occurred in the active condition In no case, however, have these protozoa been able to reduce the bacterial contents of their respective soils to a level permanently below that of the toluened soil Cultures were made at the end of the experiment to ascertain if protozoa were present in the toluened soil, with the result that about 5000 flagellates and about 10 amœbæ per gramme were found in it Now the curve for the toluened soil is quite a normal one, and shows the usual partial sterilisation results when compared with that of the untreated soil

Whatever be the limiting factor eliminated by the process of toluening and heating this soil, resulting in the rise of the bacterial content from 10,000,000 or 12,000,000 to 50,000,000 or 60,000,000 bacteria per gramme, that factor evidently has no connection with the flagellates, for these have resisted the action of the antiseptic and heat and, though much reduced in numbers at the beginning of the experiment, have succeeded in repopulating the soil

The results obtained from these inoculated soils accord with those obtained from inoculated samples of 1846 soil In those it was found that the ciliates, amœbæ, and flagellates failed to reduce the bacterial content below the level of the untreated soil In this soil they have not brought down the numbers of bacteria lower than those of the toluened soil. The only inference which I can draw from these results is that the protozoa have not functioned as the limiting factor on bacterial activity

The curve representing the counts for T. + Bacteria as compared with those for the toluened and untreated soils is shown in fig 7. It is

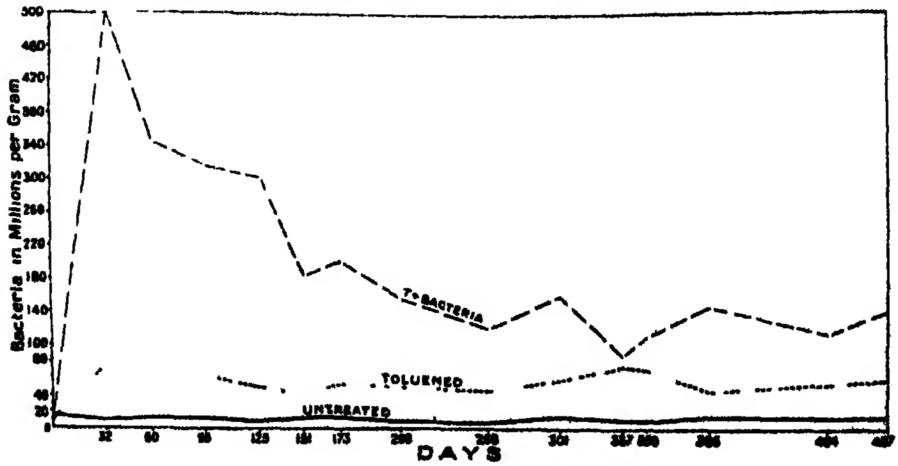

FIG 7

evident from this that the bacterial content of the T + Bacteria has maintained a consistently high level, the numbers never going down to those of the toluened soil, though there is a decided drop between 32 days and 337 days It might, at first sight, be supposed that high bacterial content was due to the absence of protozoa from the T. + Bacteria soil, the bacteria of the soil left after treatment, together with those added, having no preying organisms around them to check their growth The T + Bacteria soil does, however, contain protozoa, no doubt the offspring of those which withstood the partial sterilisation treatment, to the extent of about 3000 flagellates and 100 amœbæ per gramme Thus there are almost as many flagellates and many more amœbæ per gramme of this soil than in the toluened soil. Yet in spite of these numbers of protozoa the bacteria have maintained a much higher level than those in the toluened soil

The high bacterial counts obtained during the first 160 days in all the four soils, viz T + Bacteria, T + Ciliates T. + Amœbæ, and T + Flagellates, together with the decided drop in all cases, are very interesting At first sight it might be assumed that in the case of the three soils inoculated with protozoa the drop was due to the limiting action of the latter becoming well established This would appear to be sound reasoning if it were not for the fact that a similar drop occurs in the T + Bacteria soil, where no protozoa were added Moreover, the protozoa found at the end of the experiment in the T + Bacteria and in the toluened soils are quite comparable, and if we were to assume that the drop in the bacterial content in the T + Bacteria soil was due to the activity of the protozoa surviving partial sterilisation, we should be confronted with the difficulty that in one soil the surviving protozoa were exerting a limiting action, whilst in the other they were not doing so, though conditions for trophic existence were equally good in each case The high counts during the first 160 days may probably be explained by the fact that hay-infusion and very large numbers of bacteria were added to the soils in inoculating them and in this way the conditions brought about were very favourable to extreme bacterial activity as compared with the toluened soil, to which only sterile distilled water was added, and which consequently exhibits no exceptionally high bacterial figures In the same way the fall in the bacterial counts after about 160 days in these soils may, perhaps, be accounted for by assuming that the food supplies added with the hay-infusion became exhausted, and as a result of this the bacteria dropped to somewhere near the level of the numbers present in the toluened soil

Fig. 8 represents the curve for the T. + 5 per cent untreated soil along with those for the toluened and untreated soils It is evident that after

151 days the bacterial content of the inoculated soil remained at a lower level than that of the toluened soil At the same time it appears to be pretty

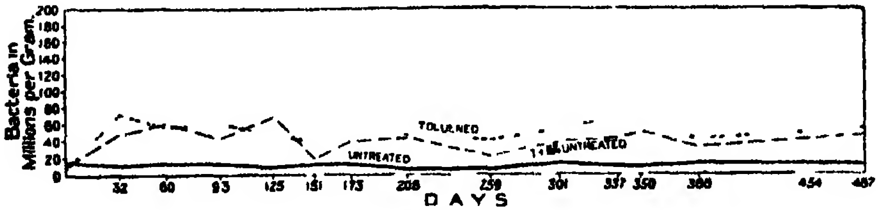

FIG 8

clear that the two curves are of the same order The T + 5 per cent untreated does not show a continuous and persistent decline in the numbers of bacteria, as one would expect if the limiting factor were due to the growth and activity of protozoa added with the untreated soil The two curves are practically parallel from 259 days onwards, and it is obvious that the same set of conditions was affecting the bacteria in each soil

The numbers of protozoa present in each soil are as follows —In the T. + 5 per cent. untreated there are at least 10,000 flagellates, about 500 amœbæ, and an almost negligible number of ciliates—5 or 10—per gramme * In the toluened soil there are about 5000 flagellates and about 10 amœbæ per gramme There are thus very many more protozoa per gramme in the inoculated soil than in the toluened soil, and the lower bacterial content of the former soil is thus easily accounted for if we assume that the protozoa act as the limiting factor If we only had these two curves and that for the untreated soil on which to base our conclusions, the above inference might be considered correct. But when we take into consideration the points already mentioned in connection with the other inoculated soils, it is scarcely possible to assume that this is the real explanation.

It has been shown that in the case of the toluened and the T. + Bacteria soils that the flagellates can be left out of account, so far as any possibility of functioning as the limiting factor is concerned So that if the lower bacterial

* This soil affords evidence of the activity of the amœbæ added in the 5 per cent. of untreated soil. Amœbæ are present in the latter to the extent of about 3000 per gramme, and assuming that this number was present at the time the soils were mixed, we can reckon that in each 100 grm. of the mixture there were 15,000 amœbæ or 150 per gramme. There were present at the last protozoal count 500 per gramme, thus showing that the original 150 had increased to 500 per gramme. Similarly the 1846+10 per cent. 1870 soil discussed on p. 447 gives evidence of the amœbæ added in the 10 per cent. of 1870 soil having increased from 50 per gramme at the beginning to 2000 per gramme at the end of the experiment.

content of the T + 5 per cent untreated soil is due to protozoal activity it must be the 500 amœbæ and about 10 ciliates per gramme which are responsible for it It seems to me highly improbable that this is the true explanation when we consider the enormously larger numbers of amœbæ and ciliates present in the T + Amœbæ and T + Ciliates soils, where the protozoa obviously have not effected a limiting action on the bacterial contents of their respective soils

It is clear, however, that some factor has been added to the toluened soil in the 5 per cent. of untreated which acts as a check on bacterial growth I cannot find support in these results, however, for the assumption that this limiting factor is the protozoa I would suggest that the influences checking the growth of bacteria are connected with some other property of the added soil than its contained protozoa.

General Discussion.

The introduction of large numbers of bacteria into the samples of soil along with the added protozoa must be a source of disturbance to the bacterial flora, and for this reason the experiments dealt with above cannot be considered as showing a clear issue between protozoa on the one hand and bacteria on the other

I sought to reduce this source of error to a minimum, however, by the continuation of the experiments over a long time, thus allowing the disturbed bacterial floras to settle down so that any influence of the protozoa should be judged after this steady point had been reached, *i e* after about 160 days in both the 1846 and the Hoosfield soil

In order that the protozoa should have conditions, as near as I could bring them about, favourable to excystation I partially air-dried all the soils after they were inoculated

In this way I hoped to meet the criticism which might be brought against the experiments that the protozoa had failed to function Moreover the soils were all kept under conditions of temperature, water-content and aeration exactly comparable with those under which Russell and Hutchinson kept their soils. If protozoa therefore could act as they supposed them to do in their soils they had every chance of doing so in my soils.

Another point calls for some comment. Martin and Lewin* have found evidence of an abundant fauna of active amœbæ and flagellates devouring bacteria in certain soils which they have tested. They suggest that these have probably some influence on bacterial numbers and thus on soil fertility Their results are very important direct evidence of the activity of protozoa in

* "Some Notes on Soil Protozoa," 'Phil Trans.,' B, vol. 205, pp. 77–94 (1914)

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the soil, but this does not prove that the amœbæ and flagellates are functioning as the limiting factor in the sense in which that term is used by Russell and Hutchinson

Before this can be shown to be true it will be necessary to correlate protozoal activity with a decrease in the numbers of bacteria in a given soil specially inoculated with protozoa.

I have shown above (p. 446) that the presence of 10,000 amœbæ per gramme of soil is not sufficient to reduce the bacterial content of a soil to the level of a similar soil containing no protozoa even though the soil be kept under conditions of moisture, etc, favourable to the trophic existence of amœbæ and flagellates

Conclusions

The results of the experiments described above lead me to the conclusion that the protozoa, including ciliates, amœbæ, and flagellates, added to the soil have not been able to act as a factor limiting bacterial activity in the soil.

Inferentially, therefore, the ciliates, amœbæ, and flagellates obtainable from ordinary soil under cultural conditions do not function as the limiting factor.

This is in accord with and extends the conclusion put forward in my earlier paper,* viz., that the ciliated protozoa are present in soil only in an encysted condition and cannot function, therefore, as the factor limiting bacterial activity

There is evidence, however, in the case where a small quantity of untreated soil is added to a partially sterilised soil that some factor comes into action which keeps down the level of the bacterial content The results obtained, however, do not lend support to the hypothesis that it is the protozoa added in the untreated soil which have this influence.

It is shown in the case of Broadbalk 1865 soil, in which an abundant protozoan fauna of amœbæ and flagellates is present, and presumably active, that the numbers of bacteria maintain a high level This soil exhibits a clear case of partial sterilisation being effected without the elimination of protozoa.

It is not within the province of this paper to attempt to rebut the very weighty indirect evidence put forward by Russell and Hutchinson as to the biological character of the detrimental factor. The results obtained, however, warrant the conclusion that ciliates, amœbæ, and flagellates cannot be included in that biological factor

* 'Roy Soc. Proc,' B, vol 84, p. 165 (1911).

On the Mesodermic Origin and the Fate of the So-called Mesectoderm in Petromyzon

By S Hatta (Sapporo, Japan)

(Communicated by Prof E W MacBride, F R S Received December 10, 1914)

Introductory.

About 20 years ago v Kupffer (85) described in the embryos of *Petromyzon* an epithelial structure extending, between the ectoderm and the somatic plate of the mesoderm, from the head to the posterior boundary of the branchial region, and described it under the name of the neurodermis, subsequently, he bestowed on it the name branchiodermis Seventeen years later the same structure was again discovered by Koltzoff (02), who identified it with the mesectoderm which was described by Miss Platt (94) in *Necturus* embryos Subsequently, so far as *Petromyzon* is concerned, nothing was published until last year, when a paper by Schalk (13) appeared, although the corresponding layer of cells was described by A Dohrn (02) in Selachii and by Brauer (04) in Gymnophiona

For a long time the origin and fate of the layer in question engaged my attention Last summer I was able to re-examine my sections and to confirm observations which I had previously published in a paper entitled "Die Bildungsweise und erste Differenzierung des Mesoderms beim Neunauge (*Lampetra mitsukurii*, Hatta)," in which the origin and differentiation of the so-called mesectoderm are described and illustrated by a series of microphotographs To my regret the paper, which was ready for press when the great war broke out, could not be sent to the editor of a certain scientific journal in Belgium, who had promised to publish it in his journal The present note is an attempt to communicate some of the principal points of that paper which relate to the mesectoderm The other organs dealt with in the above-mentioned paper have already been described in preliminary notes or in my previous papers

1. *Origin of the Mesectoderm.*

The previous authors who deal with mesectoderm invariably assume its ectodermic origin The layer was so named by Platt, because, in spite of its supposed ectodermic derivation, it takes on in its further differentiation features like a mesodermic structure. About the mode in which the layer arises from its assumed mother-layer, positive evidence has not as yet been given by any of the authors, except Schalk, who endeavours to make

intelligible, by means of figures and descriptions, the precise mode in which it originates

According to v Kupffer and Koltzoff, the mesectoderm is formed of cells liberated partly from the medullary cord, but in the main from the ectoderm forming the lateral walls of the head and of the branchial section of the body. The cells of the branchial region are regarded by them as being proliferated from the ganglionic placodes These cells, whether medullary or ectodermic in origin, are classed together by the authors and designated as *ectodermal*, because the cells from the two sources become so much intermingled as to be indistinguishable, when once they have left their mother-layers

But the cells of the mesectoderm become differentiated, as their later history shows, in two directions viz. into the cephalic nerves with their internal ganglia on the one hand, and into the tissues which give rise to the cartilaginous branchial basket and the connective tissue on the other For this reason probably the mesectoderm has been designated either as neurodermis or as branchiodermis, until Koltzoff (02) identified it with the corresponding structure found by Platt in *Necturus lateralis* Neither v Kupffer nor Koltzoff were able to distinguish in the mesectoderm the nerve cells from the other elements, but each states that both the nerves and connective tissue are derived from one and the same source.

Koltzoff distinguishes in the mesectoderm the dorsal division, which is found above and within the cephalic ganglia, and the ventral division, which extends below them towards the ventral surface This attempt amounts to nothing and is founded only on histological distinction, the dorsal division is a network of the strings caused by the connection of the cells with one another by their protoplasmic processes, while the ventral division of the layer consists of a typical epithelium of columnar cells But this histological difference is a temporary one, and does not indicate, as Koltzoff believes, a differentiation of the nerve cells from the other elements of the mesectoderm.

The principal point in which the results by Schalk differ from those of the authors above mentioned consists in the origin of the mesectodermal cells, which, according to him, is confined absolutely to the ectoderm, he denies positively that any of these cells have a medullary origin. He says, however, nothing definite about the nerve cells or the mesoderm somites, except that the sclerotomes give rise to the trabeculæ and parachordals.

If I understand Schalk correctly, there are two phases in the liberation of the mesectodermal cells from the ectoderm. In embryos about ten days old selected from his material, the heads of which have just begun to be raised

above the yolk, more or less conspicuous groups of cells are proliferated from the ectoderm, at about the level of the chorda and uninterruptedly from the eye backwards along the whole extent of the branchial tract of the enteric canal, and these cells push their way ventrally between the ectoderm and mesoderm The cells of this first phase are, the author believes, identical with those of the branchiodermis of Kupffer

In quite young embryos the production of these cells goes on throughout one continuous streak, but in a little more advanced stage it is concentrated in certain centres, which Schalk believes to have been detected by him and which resemble the nerve placodes of Kupffer This concentration indicates the beginning of the second phase of cell production

In the second phase of the formation of mesectoderm which Schalk describes, each centre of cell production is found close behind each visceral pouch, except the hyomandibular, which is destitute of such a centre The statements are illustrated by his text-figs. 16 and 17 The centres are produced by local thickenings of the ectoderm, which appear from before backwards one after another and proliferate the cells in a continuous layer, which becomes pushed backwards so as to be mixed with those of the branchiodermis, so that the cells of both lots can no longer be distinguished one from the other But he says nothing definite as to whether the branchiodermis, or the cells directly descended from the placodes, represent the formative elements for the branchial cartilage bars He says merely quite indefinitely "Wenn nun auch jene kleinen Plakoden moglicherweise an der Bildung der Branchialnerven Anteil haben, so glaube ich doch behaupten zu konnen, dass ein Teil der aus jenen Epidermisplakoden auswandernden Zellen bei der Bildung der Kiemenknorpel verwandt werden" (14, p. 55).

Schalk is correct in so far as he excludes the medullary cells from the origin of the mesectoderm, in other respects the results given by him do not add anything to what everybody had assumed

I have observed all the occurrences which Schalk gives in his figures and found that they have nothing to do with the mesectoderm.

How and when the cell proliferation of the placodes in the branchial region is closed, we cannot learn from the statements given by him at all But his text-fig 18 shows a great resemblance to a section from a series of frontal sections through an embryo, 12 to 13 days old, in my possession, which is just hatched or is about to break the chorion. At such a stage as this the mesectoderm is, of course, already fully established and has commenced even to be differentiated, as is clearly seen in the figure referred to; the continuous epithelium is divided by the outgrowth of the visceral pouches into

branchiomeres, and the proximal part of each branchiomeric piece is swollen up (Schalk's text-fig 18, p 6), giving rise to the cartilaginous visceral bar * The ectoderm lying in close contact with the thickened part of the mesectodermal epithelium, which is shown in the figure by Schalk as continuous with the ectoderm, is a thickening produced by active multiplication of the component cells of the ectoderm (text-fig 1, A).

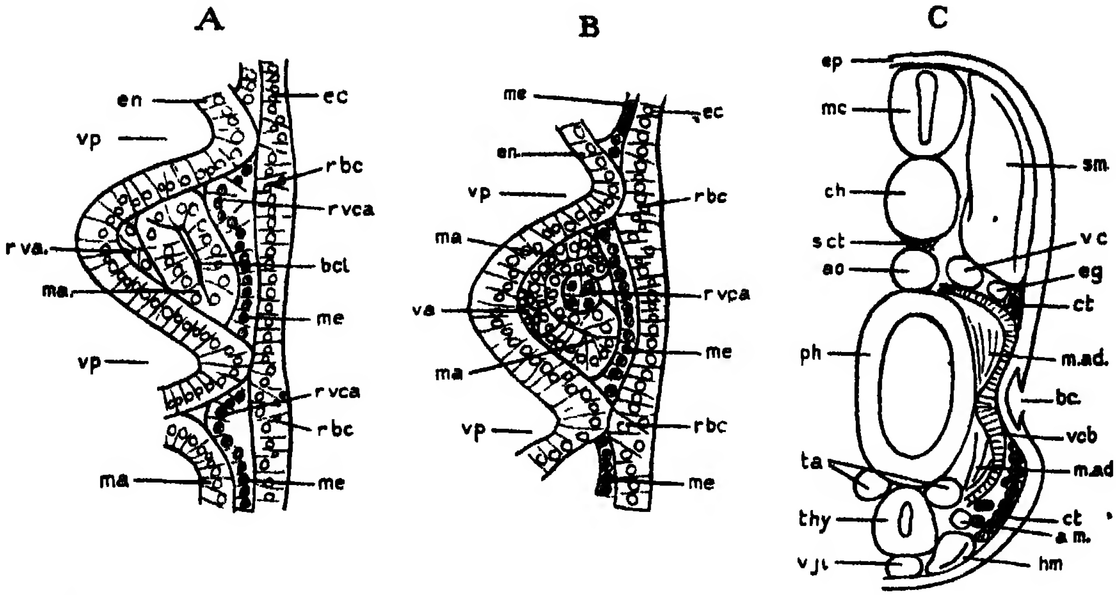
This ectodermal thickening assumes an oval outline with its long axis vertical and grows inwards (text-fig 1, B), pressing against the rudiment of the cartilaginous visceral bar But the conical bottom of the entodermal visceral pouch in front pushes its way laterally and backwards, and presses upon the invaginating ectodermic pouch, finally fusing with it On the 14th day this spot becomes perforated and forms an oval slit with its long axis vertical, and the gill slit is thus established (text-fig 1, C) The rudiment of the cartilaginous visceral bar is found close behind this orifice Now, the thickening of the ectoderm which Schalk saw was the developing gill-cleft and had no genetical relation to the cartilaginous visceral bar.

A thick section might induce one to assume continuity of the thickened ectoderm and the likewise thickened mesectoderm, but, in reality, both the parts are separated by a sharply defined boundary line, as careful observations of thin sections prove without difficulty.

In spite of his efforts, Schalk could not detect, as he says, a corresponding placode in the hyoid segment Here, in fact, no ectodermal thickening for the gill-cleft takes place at all, because the hyomandibular pouch in front of this visceral area does not break out to the exterior, but is transformed into the velar cavity; it has no direct respiratory function, but performs an auxiliary service to it.

I may be permitted to add a few words on the placodes of nervous nature in the branchial region, in order to avoid possible confusion of them with the ectodermal thickening for the gill-cleft above stated V. Kupffer (94) was the first who described the ganglionic placodes, termed by him the epibranchial placodes The placodes of the epibranchial ganglia are situated at the level of the dorsal edge of the lateral plates, consequently at a much higher level than that at which the ectoderm thickens for the gill-clefts, and the placode appears in each branchiomere from the vagus segment, which represents the fourth branchiomere, counting from the premandibular arch to the hindmost branchiomere behind the last or eighth visceral pouch. These placodes are, of course, purely nervous and have nothing to do with the mesectoderm, they represent the posterior section of what I have called

* The cartilaginous visceral bar in *Petromyzon* is not to be confounded with the true visceral arch formed in higher craniota.



TEXT-FIG 1 —A and B show frontal sections, of which B shows a little further advanced stage C represents a transverse section through a postotic visceral arch of a much advanced larva The nuclei of the mesectoderm cells and the structures derived from the mesectoderm are shaded *a m*, muscular artery, *ao*, aorta, *bc*, branchial cleft, *bcl*, branchiocœle, *ch*, chorda, *ct*, connective tissue, *ec*, ectoderm, *eg*, epibranchial ganglion, *en*, entoderm, *ep*, epidermis, *hm*, hypoglossal muscle, *ma*, mesodermal visceral arch, *m ad*, adductor muscle, *mc*, medullary canal, *me*, mesectoderm, *ph.*, pharynx, *r bc*, rudiment of branchial cleft, *r va*, rudiment of vascular arch, *r vca*, rudiment of visceral cartilaginous arch, *s ct*, subchordal connective tissue, *sm*, scleromyotome, *ta*, truncus arteriosus, *thy*, thyroid gland, *va*, visceral vascular arch; *v c*, cardinal vein, *vcb*, visceral cartilaginous bar, *vji*, vena jugularis impar, *vp*, visceral pouch

in my paper above referred to (14*b*) the ventral series of cephalic ganglia The epibranchial placodes are not only cut off from their mother-layer, but have been already transformed into the definitive nervous system of the branchial apparatus, when the ectoderm commences to be thickened for the gill-clefts

About the fate of the foremost placode, which is situated, as seen in text-fig. 14 by Schalk, immediately behind the optic cup, the author gives no definite account. Judging from the position in which it is found, and from what he says about it, the placode must be taken to be the rudiment of the lens which belongs to the trigeminal region Here the circumstances are not so simple as shown in the figure The ophthalmic and trigeminal placodes and the placode for the lens have coalesced at their bases and are distinguishable from one another only by the divergence of their distal parts, and they embrace between them the second mesodermic somite and a part of its mandibular fold, being closely apposed to one another. The

placodes are purely nervous in nature and have no genetic relation to the mesectoderm at all, although they are at certain stages in close contact with the latter In the course of the seventh day, therefore, before the first appearance of the epibranchial placodes, the placode for the trigeminal group is constricted off from the ectoderm

Finally, the origin of the branchiodermic cells in their first stage, of which Schalk speaks, seems to be unintelligible Judged from his text-fig. 12 and the accompanying statements, the cells are brought into their position from the ectoderm not by cell-multiplication going on in this germinal layer but simply by liberation of some of those composing the layer. If such a case as given by Schalk really occurs, it might be looked upon as a case of delamination But the occurrence of delamination, even for the formation of the ventral parts of the mesoderm, as W. Scott (82) and, later, Mollier (06) assume, or for the origin of the pericardium and endocardium, as asserted by Shipley (87), has been disproved, and, according to my experience, occurs in no case at least in the development of *Petromyzon* In the series of sections in my possession I find no similar case to that of Schalk, except some sections frontally cut through the lower margin of the well-established mesectoderm

According to the results obtained by myself the so-called mesectoderm is not so peculiar a structure as it appeared to the previous observers, but it is the mesoderm itself, a part of the somites or, as we may term them, the scleromyotomes It is represented at its first appearance, as observed in the mandibular arch, by scattered free cells, which later coalesce for the most part, to form a typical epithelium In the postotic region the mesectoderm is, on the contrary, from the first epithelial

In early stages there are found only two kinds of free cells. the blood vascular cells and the mesectoderm cells. The cells of both kinds appear almost at the same stage, at about the fifth day from the fertilisation a few vascular cells are to be observed in the space below the chorda, and between the floor of the pharynx and the ectoderm which represents in these early stages the ventral wall of the body *

On the contrary, the mesectodermic cells, the earlier traces of which are seen already to the close of the fourth day, appear as a rule between the ectoderm and the somatic plate of the mesoderm When established the mesectoderm is confined to the head and the branchial extent of the body.

What interests us is that the mesectoderm is in the postotic region nothing else than the ventro-lateral edge of the scleromyotome which has

* As to the full account on the characteristics of the blood vascular cells and on the development of the vascular system I refer to my other papers (00, 07, 14a).

grown downwards by active cell-multiplication (text-fig 2) The growth is produced not by the rearrangement of free cells cast off, but by the outgrowth of a continuous epithelium a single cell thick, which pushes its way between the somatic layer of the lateral plates and the ectoderm until the ventral edge of this epithelium reaches the mid-ventral line of the thyroid groove The epithelium thus produced is what is called the mesectoderm

The downward growth of the mesectoderm can readily be traced step by step On the fifth day, where the growth of the layer begins, the ventro-lateral edge, for instance, of the fifth scleromyotome* is produced a short distance downwards and is wedge-shaped in cross-section The cutis layer of the scleromyotome passes over into the muscle plate round the apex of the wedge On the sixth day the mesectoderm in its anterior portion is so broad that its lower margin is found as low as the thyroid groove, while farther backwards the layer is narrowed so that it is represented in the seventh scleromyotome by a short wedge-shaped process of the latter It is only in the course of the eighth day that the mesectoderm is fully established in the posterior branchial region

The formation of the mesectoderm is, therefore, commenced in the anterior region and goes on backwards (text-fig 3) In its early stages of formation the mesectoderm is an epithelium composed of flattened cells, but it thickens gradually as its component cells assume a tall columnar character which is, doubtless, brought about by their mutual pressure resulting from repeated cell-multiplication within the layer

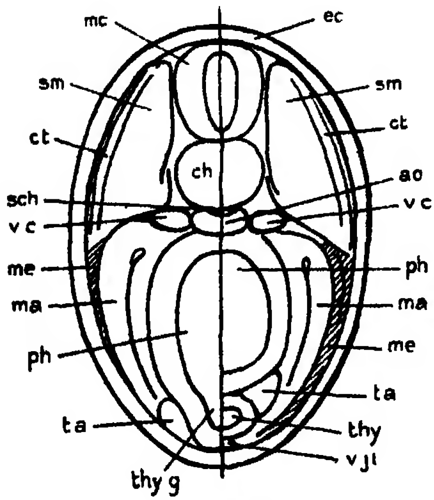
On a cross-section through a visceral arch six layers of epithelium are now seen the innermost is the entodermal pharynx wall, the next outer feeble layer represents the first rudiments of the vascular arch, then follow the splanchnic and somatic layers of the lateral plates representing the mesodermic visceral arch, and between the latter layer and the outermost layer, the ectoderm, intervenes an intensely stained epithelium of tall columnar cells, which represent the mesectoderm The mesectoderm is gradually diminished in thickness from the level of its middle height downwards into its sharp-edged lower margin.

Although the mesectoderm is only a single cell thick in most parts, at the dorsal edge, where it is in connection with the ventro-lateral edge of the scleromyotome, it is divided into two layers which pass over into the cutis layer and the muscle plate layer of the scleromyotome respectively The mesectoderm can, therefore, be regarded as a fold arising from the above-

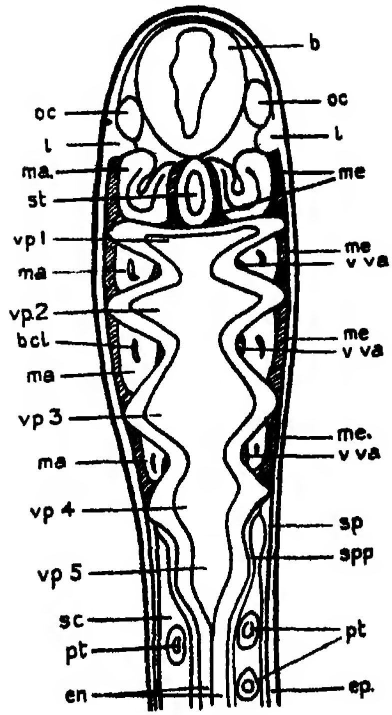
* The anterior three mesodermic somites found in front of the auditory vesicle are not transformed into typical scleromyotomes; nevertheless they are reckoned as such The fifth scleromyotome is accordingly the second postotic

mentioned edge of the scleromyotome, and wedging itself in between the meso- and ectoderm

The manner in which the mesectoderm develops reminds us of the folding



TEXT-FIG 2



TEXT-FIG 3

TEXT-FIG. 2.—Diagrammatic representation of a transverse section through a post-branchial visceral arch, showing down-growth of the cutis-layer of scleromyotome to give rise to mesectoderm (left) and the established mesectoderm (right) The mesectoderm and its rudiments are shaded *ct.*, cutis layer, *sch*, subchordal cells; *thy g*, thyroid groove. For other letters, see the explanation of the previous diagram.

TEXT-FIG 3.—Diagrammatic representation of a frontal section showing the position of the mesectoderm in relation to other layers The posterior visceral pouches are in formation, and the lateral plates are being cut into the mesodermic visceral arches. The mesectoderm is shaded *b*, brain; *bcl*, branchiocœle, *ep*, epidermis; *l.*, placode of lens, *ma*, mesodermal visceral arch; *me*, mesectoderm, *oc*, optic cup; *pt.*, pronephric tubule, *v va*, rudiment of vascular arch, *sc*, splanchnocœle; *spp.*, splanchnopleure, *sp*, somatopleure, *st*, stomodæum, *vp*, visceral pouch

of the corresponding edge of the scleromyotomes in the postbranchial region, by means of which the scleromyotomes grow downwards so as to provide the ventral somatic walls of the body with the muscular and the cutis layers. The downward growth of the scleromyotomes in both cases is, I venture to

assume, analogous, and the two structures thus brought about are serially homologous The difference consists in that the mesectoderm is destitute of the muscle plate layer Though to a very small extent, the mesectoderm is divided into two layers, and there can be no objection to our regarding the inner of these as homologous with the muscle plate

The causes of this modification in the branchial region are to be sought in the changes in that region produced by the respiratory mechanism and its skeletal framework. As will be stated later on, the mesectoderm is converted to a great extent into the cartilaginous branchial bar, which is a special skeletal arrangement for the respiratory mechanism The remainder of the layer supplies the elements for the subcutaneous tissue, while, in the postbranchial region, the whole of the cutis layer is employed in the formation of this tissue The demand for the formative elements of this tissue has caused, as I believe, the cutis layer of the scleromyotomes in the branchial region to be developed so vigorously as to call the mesectoderm into existence, although the muscle plate in the branchial region is almost entirely suppressed The consequence of this suppression is that the somatic walls of the branchial chamber are destitute of the segmental muscles, and have to fill this deficiency by the so-called hypoglossal muscles which with their cutis layers undergo an exceedingly modified mode of development,* as was pointed out by Neal (97) and was confirmed by Koltzoff (02),† and by myself (14*a*, 14*b*)

The great modifications met with in the prootic section of the head cause the mesoderm to be modified to a still greater extent than in the postotic branchial region Accordingly some peculiarities occur in the formation of the mesectoderm in this region

In this section of the head there are formed three mesodermic somites, the third being situated just in front of the auditory vesicle Of these three somites the hindermost shows a structure very similar to a postotic somite, the middle somite, under which the lateral plates‡ develop into the enormous mesodermic mandibular arch, is represented by a narrow epithelial fold of the archenteric roof, which ascends along the lateral wall of the medullary canal and strikes with its distal extremity against the trigeminal placode of the

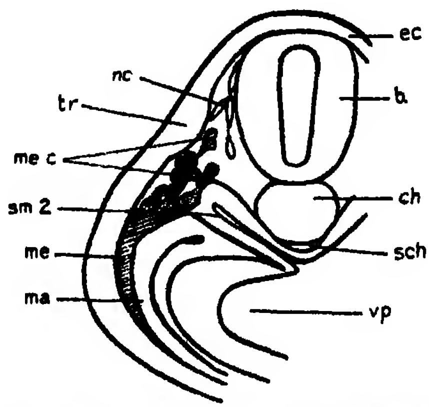
* The hypoglossal muscles are produced by the forward bending and shifting of the ventral part of some postbranchial scleromyotomes. They are the only segmental muscles in the somatic walls of the branchial chamber.

† As Koltzoff (02) remarks, Goette (90) gives incorrectly four somites in front of the auditory vesicle.

‡ Koltzoff is the only author who gives a detailed account of his second somite But he failed to detect the free somite, which is very small, whilst he regards the dorsal part of the colossal lateral plates as their somitic part.

ectoderm; whilst the first somite is represented by the anterior blind end* of the epithelial archenteron, which is folded off from the rest as a median unpaired pocket This somite is so small that it is practically destitute of the lateral plates.

In the two posterior of the three somites the outer wall, which corresponds to the cutis layer of a postotic somite, gives off free cells which push their way between the ectoderm and the somitic layer of the lateral plates (text-fig 4) The free cells are quickly increased to some extent by the cells



TEXT-FIG 4 —Diagrammatic representation of a transverse section through the second somite, showing the proliferation of the mesectoderm cells from the lateral layer of the somite *me c*, mesectoderm cells (shaded), *nc.*, nerve cells, *sm.2*, second somite standing still in connection with the pharynx, *tr.*, placode for trigeminal ganglion. For other letters, see the explanation of the previous diagrams.

budded out from the lateral layer of the somitic fold The somites are cut off from the archenteric roof only on the fifth day, while the cell-proliferation begins at the early part of the fourth day, therefore earlier than the stage at which the ventro-lateral edge of the postotic scleromyotomes begins to be produced into the mesectoderm The free cells soon make up a thick columnar epithelium which represents the mesectoderm of this preotic region In contrast to that in the postotic region, the mesectoderm is here not confined to the lateral part, but is spread into the ventral wall of the body, which gives rise to the stomodæum by invagination

At the same time, but on a smaller scale, the cells wander out of the lateral layer of the somites into the space between the medullary canal and the ectoderm opposite and above the ganglion placodes. They do not assume

* The first somite may not be confounded with the anterior blind sac of the pharynx of Kupffer; for the explanation of both the structures I refer to my paper above given (14*b*).

an epithelial character, but make up a simple network of cells with the nervous cells coming forth from the nerve ridge, by which the epidermic ectoderm is connected with the walls of the medullary canal This network corresponds to that part of the mesectoderm which Koltzoff distinguishes as the dorsal division from the epithelial ventral part of it The dorsal division is accordingly confined to the preotic region, while the ventral division is to be traced uninterruptedly to the corresponding part in the postotic branchial region and constitutes one continuous structure from the mandibular arch to the hindmost visceral arch

The network forming the dorsal division becomes gradually more complicated, owing to further growth of both the nervous and mesectodermic cell-strings, so that the elements of both kinds are not easily distinguished from each other, as Koltzoff complains The cells of the dorsal division ought not, therefore, to be overlooked at the earlier stage of their appearance, a phase in which the nervous cells coming downwards from the medullary roof and the mesectoderm cells arising from the lateral layer of the somites do not as yet meet with each other Then, in the following stages, both kinds of cells are not very difficult to trace into the points from which they start respectively At the dorsal corner of the lateral layer of the somites active cell-divisions can be observed which are repeated during the course of development for not less than 12 hours, and the course taken by the resulting cells in passing into the network is not difficult to make out The nerve-cell strings passing downwards almost vertically can also be traced with certainty The nerve fibres developed from these strings associate with those from the ganglion of epidermic origin and make their way between the mesectoderm and somitic layer of the lateral plates, as was obvious already in an embryo of the eighth day

The foremost somite gives off the mesectoderm cells not only from its outer wall, as in the following somites, but also from the anterior wall of the blind pocket by which the first somite on each side stands in connection with its fellow. The cells from both sources fill up the space between the ectoderm and the somite and the space below the anterior extremity of the brain. The free cells lying in close contact with the ectoderm are transformed into the epithelial mesectoderm, which can by no means be distinguished from the ventral division in the posterior region and is continuous to it; those inside are developed into the network of muscle fibres which occupies the interior of the upper lip in later stages.

While the outer walls of the first, second, and third somites and also the anterior wall of the first are broken up into the mesectoderm cells, the inner walls of these three somites, which correspond to the muscle plate of the

postotic somites, give rise to the six muscles of the eye,* which are distinctly differentiated already at the close of the ninth day or at the commencement of the 10th day The preotic section of the head is consequently totally destitute of the segmental muscles derived from the somites. This deficiency is, as pointed out by Kupffer, made good by a few myotomes behind the auditory vesicle, the dorsal parts of which are bent and shifted forwards into the head.

The mesectoderm is, therefore, the product of that part of the mesodermic somites which corresponds to the cutis layer. The dorsal division, which is distinguished by Koltzoff from the ventral division, is derived from the three preotic somites and confined to the preotic section of the head, it never assumes an epithelial structure, but makes a simple network with the nerve-cell strings

On the contrary, the ventral division is a continuous layer of typical epithelium, extending from the anterior margin of the lateral plates to the posterior boundary of the branchial region and as high as the lateral plates. This division of the mesectoderm is cut into nine vertical epithelial bands, when the lateral plates are divided into nine visceral arches

That part of the mesectoderm which assumes the epithelial character in the snout may be regarded as the anterior continuation of the ventral division. If this assumption is correct, all the three preotic somites, just like the following somite in the postotic branchial region, contribute elements to the ventral division of the mesectoderm

2. *Fate of the Mesectoderm*

While the nerve-cell strings in the pre- and postotic region are transformed into the nerves and ganglia, the mesectodermic elements of the dorsal division are converted mainly into the connective tissues standing in relation to the nerves and ganglia, a small portion of them, which lies in contact with the medullary walls, gives rise to the most anterior section of the cranial skeleton, *i.e.* the trabeculæ

The nine mesectodermic bands, into which the continuous layer of the ventral division is divided by the visceral invagination of the pharynx-walls, undergo the following differentiation. On frontal sections through a just-hatched larva, the first stage of the differentiation is very obvious. At the level of the visceral pouch, the entodermal wall of the pharynx is in close contact with the ectoderm, while at the level of the visceral arch, between the two layers, are contained the vascular cells, the mesodermic arch and the

* Detailed accounts on the development of the ophthalmic muscles I have given in my above-mentioned paper (14*b*).

mesectoderm. The proximal half of the last-named epithelium is thickened so as to be raised inwards into a ridge, which shows on cross-sections a pyramidal outline and represents the first rudiment of the cartilaginous visceral arch. This stage of differentiation is represented in text-fig 18 by Schalk, which is correct, except the connection of the mesectoderm with the ectoderm.

The cells forming the rudiment of the cartilage acquire a radial arrangement (text-fig 1, A) and are soon constricted off from the remainder of the layer, this stage is followed by a stage in which they are wedged in between one another, so as to form one row (B). On cross-sections through a larva of the 14th day this rudiment of the cartilage looks like a bar consisting of piled-up discs, in which three sections are distinguishable (C) a dorsal and a ventral section curved outwards and the middle section bowed inwards. While the ventral section touches with its distal extremity the lateral division of the vena jugularis impar, the proximal end of the dorsal section lies in the corner between the dorsal aorta and the chorda and under the anterior cardinal vein. In the course of the 15th day, the aorta together with the roof of the pharynx is separated from the chorda by enormous development of the reticular subchordal connective tissue. Accordingly the dorsal end of the rudiment of cartilage is also brought downwards, so as to be forced into the corner between the aorta and the roof of the pharynx which has been pressed down. It is interesting that the band of connective tissue which before and after this change connects the rudiment of cartilage with the chorda is drawn out into a string stretched between both the structures.

In sharp contrast to other visceral arches, the hyoid arch does not undergo this dislocation of the cartilage bar, which is, on the contrary, shifted by stages a little upwards, and the cardinal vein passes into the mandibular vein *under* the cartilage bar. This peculiar feature is the first step towards the fulfilment of the function which the hyoid arch has secondarily acquired, it enters into the formation of the primordial skull, leaving the service of respiration.

The differentiation of the mesectoderm into the visceral arch takes place at first in the visceral arch behind the hyoid arch and proceeds backwards to the following arches, which undergo the same process one after another. For a long while the mesectoderm in the hyoid arch is not cut off from that in the mandibular arch. And it delays its differentiation into the rudiment of the cartilaginous hyoid arch, which is, however, obvious before the same process commences its work in the hindmost visceral arch.

The rudiment of the cartilaginous visceral arch shifts inwards, when it is detached from the remainder of the mesectoderm, and presses and separates

at last the lateral plates, the mesodermic visceral arch, into the inner adductor and the outer constrictor muscles, with which the cartilaginous arch is invested.

The remainder of the mesectoderm constricted off from the rudiment of the cartilaginous visceral arch is stretched at the same time to the outside of the rudiment of arch so as to line the whole inner surface of the ectoderm ventral to the chorda level, and assumes the characteristic feature of the subcutaneous tissues which underlie the ectoderm, except the ventral part for the hypoglossal muscles. This differentiation of the mesectoderm is very obvious in larvæ of the 13th to the 14th day.

The mandibular arch, which we may now consider, is characterised particularly by the enormous mesodermic arch which it contains and which is folded upon itself, thrust inwards by the invaginating stomodæum, so that four layers of the folded lateral plates are obvious on a cross-section through this arch. While the ventral edge of the folded mesodermic arch, by which the somatic layer passes over into the splanchnic plate, is separated by the bottom of the stomodæum from its counterpart on the opposite side, the dorsal edge, by which the two layers of the lateral plates also run into one another, is divided only by the carotid artery from the chorda. The ventral division of the mesectoderm, following this folding of the mesoderm, is brought into the same topographical relations to the stomodæum and to the chorda.

The dorsal edge of this division of the mesectoderm is brought into contact with the lateral wall of the chorda, above the carotid artery, as is very clearly seen on the 9th to 10th day. On the 13th day the cells composing this edge are concentrated into a characteristic compact mass which is soon constricted off from the remainder of the layer. This compact cell mass constitutes the earliest traces of what are known since Sewertzoff (97) as the anterior parachordals. The rudiment of the anterior parachordal is on cross-sections wedge-shaped and looks as it produced from the lateral wall of the chorda.

On the 14th day the rudiment of the anterior parachordal can be traced as far as the branching of the facial artery from the carotid, which marks the boundary between the first and second somites, and it ends backwards rather suddenly in front of the roots of the vascular mandibular arches and of the carotids. The parachordal rudiment is most prominent at a little distance from the root of the vascular mandibular arch and grows gradually lower toward the snout, while it is decreased suddenly in height backwards.

The parachordal rudiment cannot, therefore, be distinguished genetically from the rudiment of a cartilaginous visceral arch, both the structures are,

I believe, serially homologous with one another The prominent point of the parachordal rudiment develops into a transverse bar of cartilage, which is, I assume, the rudimentary remnant of the equivalent of its corresponding visceral cartilage bar This bar is, in the mandibular arch, reduced to its last remnant, because it has been shut off from the respiratory mechanism The further fate of the cartilage bar interests us in developing into that important element of the primordial skull, which is known since Parker (83) as the palato-quadrate, the redevelopment of this rudimentary remnant into so conspicuous an element of the cranial skeleton is due to nothing but the law of "Funktionswechsel" first enunciated by Dohrn

The remainder of the mesectoderm detached from the rudiment of the anterior parachordal is, in the mandibular arch as elsewhere, converted into the subcutaneous tissues, which develop in distinction to those in other arches not only beneath the ectoderm, the skin of the cheek, but also beneath the epidermis of the stomodæum, the cover of the mouth cavity

As Gaup (06) remarks, the single origin of the parachordal, which Koltzoff (02) maintains and Schalk (13) confirms, is incorrect On the contrary, the posterior parachordal of Sewertzoff (97) is represented in reality by the medial horizontal process of the cartilaginous hyoid arch itself, and the transverse process, which, according to Sewertzoff, is very similar to an ordinary visceral bar in the following arches, is nothing more than the visceral bar itself in the hyoid arch

The anterior and posterior parachordals are separated for a long time by interposition of the large auditory capsule Both the rudiments grow respectively backwards and forwards to meet and be fused together with each other only in a larva about thirty days old, in which the auditory capsule retreats and is detached from the chorda But for a long time the transition is obvious, because both the rudiments are decreased in thickness towards their point of meeting

The trabecula is formed in front of the root of the facial artery and the rudimentary vascular arch in the premandibular segment, its centre lies close to this vascular root From this centre it grows anteriorly along the basal wall of the brain and over the posterior cerebral artery, which is the anterior prolongation of the carotid artery. The more it is prolonged, the more it diverges hand in hand with the artery from the median line, so that it lies opposite the optic cup rather on the lateral wall of the brain and embraces, with its counterpart on the opposite side from right and left, its infundibulum, and is finally lost on the lateral wall of the latter

The mesectoderm cells giving rise to the centre of the trabecula are doubtless those derived from the first somite, and are marked off from those

of the second somite by the facial artery, which appears much earlier than the mesectoderm But the forward growth of the anterior parachordal seems to be carried on largely at the cost of the dorsal division of the mesectoderm from the second somite, viz, the cells which lie close to the chorda, and to be continued uninterruptedly to the formation of the trabecula in front, so that the trabecula is practically not formed separately from the anterior parachordal, as was believed by previous observers, but as the prolongation of the latter The trabecula has, however, a special centre for itself, marked by a slight thickening in the rudiment

It is, however, still an open question, whether the trabecula is to be put in the series of the visceral arches or not But it is obvious that the rudiment of the trabecula is genetically identical with the latter, because it is formed of material identical with that giving rise to the visceral arches and because it comes forth in a metamere identical with that which has a cartilaginous visceral arch for itself

To avoid misconception, a few words may be said about the relation of the mesectoderm to the metamery of the body The epithelial bands into which the ventral division of the mesectoderm is divided are in numerical as well as topographical correspondence with the mesodermic visceral arches, that is to say, branchiomeric The cartilaginous visceral arches are derived from these branchiomeric bands, also branchiomeric in arrangement

The mesodermic visceral arches arise from two unsegmented continuous layers, the lateral plates, which are divided by nothing but evagination of the visceral pouches, which is independent of the process dividing the mesoderm into the somites This metameric repetition in the entodermal pouches has, therefore, no direct relation to the mesodermic somites at all, which represent the primary metamery of the body In spite of its being the product of the segmented mesoderm, the mesectoderm is also an unsegmented continuous layer, until it is divided by the visceral invagination into branchiomeric bands The branchiomery in this part of the meso- and mesectoderm, which is brought about in a passive way, is, therefore, not of the same value as the body-metamery

The branchiomery is furthermore in segmental accordance with the ventral series of the ectodermic placodes for the ganglia * But this series of ganglia is never in direct segmental connection with the mesodermic somites.

Still another organic system, which is in segmental accordance with the

* This series of ganglia consists of the facial and glossopharyngeal ganglia and of the series of the epibranchial ganglia The placode for the lens may be put in this series, for it can by no means be distinguished from the ganglionic placodes, so far as its origin and mode of development are concerned.

visceral arches, is the vascular. In the first formed vascular system, in which the arterial and venous systems are not yet differentiated, we have before us a vascular system of the Annelid type (Hatta (07), Keiser (14)), which consists of a dorsal and a ventral longitudinal vessel connected with each other by vascular rings which are repeated in each body somite (Hatta (07), (14*a*), Keiser (14)). The anterior division of this ring vessel system is represented by the vascular visceral arches (Goette (90)), and is followed by Mayer's Quergefasse in the pronephric region, while in the region still further posterior the segmental character of this series of vessels is obscure, because in this region there is no organ of segmental arrangement standing in direct relation with the vessels.

The origin of the ring vessels, whether entodermic (Goette (90)) or mesodermic (Hatta (14*a*), Keiser (14)), is by no means mesomeric, because the vascular cells are derived, even in the strictly segmented branchial region, from an organ which is not segmented in accordance with the mesodermic somites.*

The pronephros is the only organ in which the vasomery, *i.e.* branchiomery, is connected with the mesomery. But there is an incontestable fact which shows that the segmental repetition of Mayer's vessels in the pronephric region is not primary but secondary. The first pronephric artery, which is evident in a larva of the 10th day, corresponds with the 11th vasomere (counting the premandibular vascular arch as the first vasomere), and the first pronephric tubule with which the artery stands in relation, is the product of the 7th mesodermic somite† (Hatta (97), (00), (14*a*), (14*b*)), while this somite is no longer found over the first pronephric tubule, when the tubule is cut off from it.

There are two movements by which the segmental discordance between the nephromeres and the myomeres is brought about, viz., the pronephros is gradually pushed backwards by the outgrowth of the visceral pouches, the somites, *i.e.* myotomes, on the contrary, move forwards after their formation, so that a few anterior of them are pushed over the preotic section of the head to give rise to the capitis muscles. The ring vessels, *i.e.* Mayer's vessels, occur in the visceral arches as well as in the pronephros only when the visceral pouches are formed and the backward movement of the pronephros has already been carried out. The vasomeres are thus put secondarily in relation to the pronephros, although the latter

* According to Goette, the vascular cells (in branchial region) are derived from the enteric wall, according to my results, which are confirmed by Keiser, the cells are derived from the mesodermic visceral arches.

† In my papers formerly published (97, 00) the first pronephric tubule is regarded as the product of the fourth scleromyotome, which corresponds to the seventh, when the three preotic somites are counted in.

is the descendant of the somites In short, the branchiomery and mesomery are independent of each other

The cartilaginous visceral arches and their equivalents in the primordial skull are branchiomeric organs, and have no genetic relation to the mesomeres

Therefore, it seems extremely probable that the participation of the sclerotomes in the branchial elements and in their equivalents, which is maintained by Koltzoff, Schalk, and others, is, if such actually occurs, nothing but accidental.

I wish to express my warmest thanks to Prof MacBride, of the Imperial College of Science, London, and to Dr. Gadow, Prof Gardiner, Mr Doncaster, and Prof Punnett, of Cambridge University, for the courtesy which they showed me during my stay in Cambridge, and especially in according to me the privilege of working there

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On the Variation in the Growth of Mammalian Tissue in Vitro *according to the Age of the Animal*

By Albert J Walton, M S, F R C S, M.B, L.R C P, B Sc.

(Communicated by Prof W Bulloch, F R S. Received December 8, 1914)

(From the Bacteriological Department of the London Hospital)

[Plate 15]

In a previous communication* it was shown that there was considerable variation in the value, as a culture medium, of plasmata taken from different animals of the same species, that these plasmata did not vary as to whether they were homogenous or autogenous, but that some plasmata were good media and some were bad During this investigation certain evidence arose that this difference might in part be due to the age of the animal

In the present investigation a series of experiments was carried out to show what was the effect, if any, of the age of the animal upon the plasma as a culture medium, and upon the tissues as regards the power of growth Carrel, Burrows, Harrison, and Ingebrigtsen have shown in several papers that embryonic tissue tends to grow more rapidly and more vigorously than adult tissue There appears to have been, however, no work conducted on the characters of the plasma, although it has been frequently assumed that the plasma of the young or embryonic animals makes a more suitable medium than that of adults, nevertheless it was permissible to believe that the reverse might in fact be true, and that the plasma of young animals is a less suitable medium It would appear important that this point should be settled, that thereby evidence might be gained as to the controlling influences on the growth of young tissue *in vivo*

Technique

The following experiments were carried out entirely with the tissues of rabbits As far as possible animals were used that had been bred in the laboratory, so that the exact age was known This was the case with all the young animals In certain cases, however, adult rabbits were bought of unknown age, but in such cases they were all fully grown and therefore could be used as adult animals As far as could be judged, they were all over a year old. The technique of Carrel was rigorously adhered to, the tissues being grown in pure plasma so that the characters of the growth might be

* 'Roy. Soc Proc,' B, vol 87, p 452 (1914)

unaffected by the presence of any stimulating or inhibiting substance. In the majority of cases two tissues were used, so as to lessen, as far as possible, any experimental errors A few cultures were made of the spleen, but most of the experiments were carried out with thyroid and liver These tissues as a rule grow well, and the growth is not obscured by the emigration of cells, as so often happens when spleen is used

Young testicular tissue was not cultivated in the present series of experiments, for it was considered that, as this tissue only fully develops later in life, false conclusions might be arrived at if the immature testicle of the young rabbit was used In the majority of cases fresh tissue was made use of, and in this case cross experiments were generally performed, the tissue of young and old animals being grown in plasmata of both animals. A certain number of experiments were conducted with stock cultures of adult testicle which had been growing for ten generations in a medium of plasma and tissue extract

(1) *Cultivations of Splenic Tissue*—

Experiment 1—An adult rabbit two years old was anæsthetised The fur on the ventral surface of the body was removed, the skin sterilised, the carotid artery exposed, and the blood collected in sterile paraffined tubes placed in ice The blood was then collected from a young animal 10 days old and placed in ice-cold paraffined tubes. Both bloods were centrifugalised

The spleen was removed from the young and old animals and placed in Ringer's fluid Four cultures were made of each spleen in each plasma, so that there were four groups.

At the end of 12 hours there was good emigration of round cells in all the preparations, but it was more marked in the case of the spleen of the young animals both in the young and old plasmata At the end of 48 hours there was a well marked growth of retiform tissue, which formed mosaic-like masses in the case of the young spleen in the old plasma, but was present in a less marked degree in the case of the old spleen in the old plasma In both cases such growth was apparently absent when the young plasma was used.

Owing to the amount of round cell emigration it was difficult to estimate accurately the extent of the growth, and hence experiments with this tissue were discontinued.

(2) *Cultivation of Thyroid and Liver Tissues*—

Eleven experiments, comprising 282 cultivations, were carried out in this group. In all cases both the thyroid and liver were cultivated at the same time. By this means experimental errors were less likely, for if the results

were due to such errors they would be less likely to occur in both groups. The ages of the young animals varied from two days to two weeks, and during this limited period there seemed to be little, if any, variation in the nature of the tissues and plasmata as regards the capacity for growth. One experiment will be described in detail, the others being carried out on precisely the same lines

Experiment 2—An adult rabbit over a year old was anæsthetised The fur on the ventral surface of the body was removed; the skin sterilised, the carotid artery laid bare, and the blood collected in sterile paraffined tubes kept in ice This animal was kept anæsthetised Blood was collected from a young animal five days old and also placed in iced paraffined tubes. Its thyroid and a piece of liver were removed and placed in sterile Ringer's fluid. The young animal was killed Similar tissues were removed from the old animal The blood was centrifugalised Cultures of the young animal were made in both plasmata, as were also those of the old animal, six cultures being made in each group.

The nature of the growth was observed and at certain periods specimens were fixed and stained After 48 hours the thyroid of the young animal showed marked growth in the old plasma, but that of the old animal in the old plasma, although showing considerable growth both of the connective and parenchymatous types of cell, was definitely less than that of the young animal. On the other hand, both specimens in the young plasma showed either no growth at all or only a few cells growing from the edge of the tissue. Similar results were obtained in the case of the liver.

The above results are well shown by the following Table —

	Old plasma	Young plasma.
Old tissue	Moderate growth	Very slight growth
Young tissue	Very good growth	Slight growth.

The above experiment was performed again 10 times. The old animals were in all cases fully developed, and the majority were known to be over one year of age. The young animals varied in age between two days and two weeks The cultures were made in groups of four or six in each media and were prepared under identical conditions. By taking groups of four or six specimens it was possible to estimate more accurately the changes in growth, for in the primary cultures it is unusual, excepting perhaps in the case of the testicle, to find that all the specimens have grown to an identical extent Moreover, the percentage of successful growths in a series is in

itself evidence of value of the suitability of the media and the power of the tissue to grow The results of these experiments may be summarised as follows.—

(a) *Young Tissues Growing in Old Plasma*—It was found that growth was in all cases extensive and successful Cultivations occurred in 100 per cent of the cases

With the *thyroid*, masses of cuboidal cells were seen extending into the plasma, and between them were large numbers of branching connective tissue cells

In the case of the *liver*, the growth was also extensive, and after 48 hours large masses of the characteristic deeply staining cuboidal cells were visible together with large numbers of connective tissue cells (fig 1) Successful results were again obtained in 100 per cent of the cases In both groups the growth continued for four or five days before any signs of degeneration occurred

(b) *Young Tissues Growing in Young Plasma*—With this group there was a very marked difference There is considerable difficulty in obtaining the blood of these young animals, owing to their small size, and it was at first thought possible that the difference in growth might be due to the fact that the plasma so obtained was not in good condition It was found, however, that the results were practically constant even after these difficulties had been overcome

In the case of the *thyroid* only 8 per cent. of the specimens showed any growth, and they were thus sharply differentiated from the specimens of the same tissue growing in old plasma Even in those cases in which the growth was present it was slight in amount In no case were any cuboidal cells seen, and even after three or four days there were only present a few connective cells growing from scattered areas at the edge of the tissue This result was constant apart from liquefaction of the plasma, which in other cases has been found to limit growth That is to say, the decrease in growth did not appear to be due to mechanical causes

With the *liver* similar results were obtained In this case a larger number of the preparations showed growth, the results being positive in 26 per cent of the cases. In one specimen (Experiment 5) there was very good growth, but in three other specimens of the same series there was no growth, and such a result did not occur again in the other series It is probable, therefore, that this result was an experimental error In the remaining specimens which showed growth the extent of the growth was slight In some cases a few outgrowths of cuboidal deeply staining cells were seen, but in no case were they so extensive as when old plasma was used as a medium

In the majority of cases only a few branching connective tissue cells were visible, and they extended only for a short distance into the surrounding plasma, the difference between the amount of connective tissue growth in young and old plasma being very marked (fig 2)

(c) *Old Tissues Growing in Old Plasma*—In this group there was a moderate amount of growth, about 80 per cent. of the cultivations being successful In every experiment some of the specimens showed good growth On comparison, however, it was always seen that this growth was less than when young tissues were used

In the case of the *thyroid* a few cuboidal cells were seen growing from the edge of the tissue after about 48 hours, growth being present in 70 per cent of the specimens. After a further 24 hours connective tissue cells were visible It was found in the parallel series that not only was growth more extensive when young tissue was used both as regards the parenchymatous and connective tissue cells, but that a larger percentage of specimens showed activity

With the *liver* good results were obtained, the characters of the growth corresponding with that described in previous communications* In this series growth took place in 88 per cent of the specimens, and when present there were always to be seen masses of deeply staining cuboidal cells, but these masses were always less marked than when young tissue was grown in the same plasma Proliferation of connective tissue cells took place at a slightly later date, the cells soon growing beyond and between the masses of parenchymatous cells Here again the zone of connective tissue growth was always less marked than was the case with young tissue (fig 3)

(d) *Old Tissue Growing in Young Plasma*—This was found to be the worst combination The tissues apparently were not so active and the medium was less suitable In the majority of cases no growth took place. The tissues stained poorly and apparently died

In the case of the *thyroid* only 3 per cent. of the specimens showed any growth at all, and even in the successful cases this was extremely slight. In the majority, even at the end of three or four days, the edge of the tissue remained sharply cut. In some the plasma was liquefied, but in others this change had not taken place, so that here again the absence of the growth was not dependent upon a mechanical factor Even in the few cases where there was any evidence of growth, no cuboidal cells could be seen At most there were one or two elongated connective tissue cells to be found after a careful search, so that at first sight there was a tendency to believe that no growth had taken place

* 'The Journal of Pathology and Bacteriology,' vol. 18, p. 319 (1914)

With the *liver* there were positive results in 9 per cent of the cases, but even in these growth was extremely slight. In no case were any cuboidal or parenchymatous cells seen There was very slight growth of connective tissue, so that after three days there could be seen in a few cases several long connective tissue cells growing here and there from the edge of the tissue (fig 4) Often a portion of the original tissue stained poorly and was manifestly dead

These experiments, therefore, strongly confirmed the observations of previous workers, namely, that the tissue of young or embryonic animals shows more active growth than similar tissues taken from adult animals In addition to this, it appeared manifest that the plasma of such young animals was not nearly so suitable a medium as the plasma of adult animals Such a condition has not previously been described, and is so opposed to what one would at first sight believe, that it seemed necessary to confirm these experiments For this purpose further experiments were carried out with stock cultures of rabbit testicle These tissues had been growing *in vitro* for 10 generations in a medium composed of two parts of plasma and one part of spleen extract At this period they were growing vigorously, so that at the end of 48 hours there was a wide zone of newly formed cells surrounding the original tissue

Experiment 13 —Stock specimens of rabbit testicles as described above were cultivated in groups of four each—(*a*) in plasma obtained from a young animal 10 days old, (*b*) in plasma obtained from an adult rabbit over a year old, (*c*) in a mixture of plasma from an old animal, two parts, and spleen extract one part. At the end of 48 hours, the specimens cultivated in the mixture of old plasma and spleen extract were growing vigorously and showed a very wide zone of active cells, mainly of the connective tissue type (see fig 5). These specimens served as a control. Those growing in old plasma also showed a wide zone of cells of the connective tissue type, but the newly formed cells, as was to be expected, owing to the absence of a stimulating extract, were considerably fewer in number and did not extend so far into the surrounding media (see fig. 6)

In the case of the tissues growing in young plasma, growth was very slight. There were only a few cells growing from the edge of the tissue (see fig. 7) and in these cells but few mitotic figures were seen.

This experiment confirmed those carried out with fresh tissues, and there can be no doubt that young plasma is a less suitable medium for the growth of tissues than that of old animals. It has been previously shown* that with older animals some plasmata are not such good media as others, and

* 'Roy. Soc. Proc.,' B, vol 87, p. 452 (1914).

that in such cases the plasma is always improved if it be frozen for a period of about three days, which is very suggestive that the poor growth is due to the presence of an inhibiting substance It is probable, therefore, that in young animals, also, the inhibiting substance is present in larger quantities, and hence the plasma makes a poor medium

Conclusions

1 Growth of tissues *in vitro* affords a valuable means of investigation as to the effects of age upon growth

2 The tissues of young animals grow more rapidly and vigorously than those of adult animals

3 The plasma of young animals is a much less suitable medium for the growth of tissue *in vitro* than the plasma of old animals.

4. The unsuitability of the plasma of young animals as a medium is probably due to the presence of an increased amount of some inhibiting substance

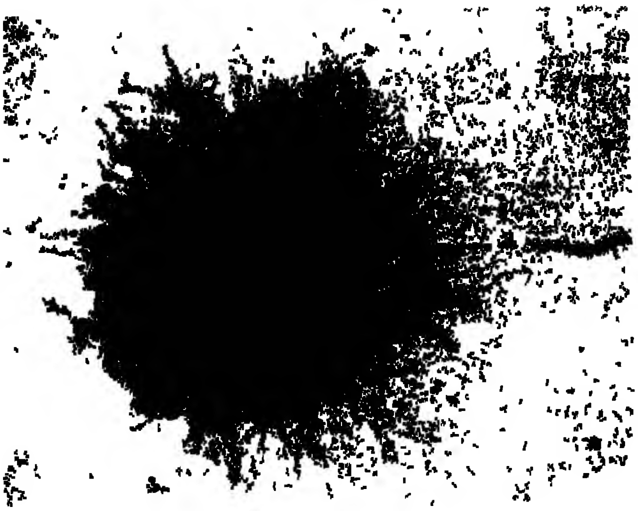
EXPLANATION OF PLATE.

Growth of Fresh Liver

Fig 1—Two days' growth of young liver in old plasma
Fig 2—Two days' growth of young liver in young plasma
Fig. 3—Two days' growth of old liver in old plasma
Fig 4—Two days' growth of old liver in young plasma.

Growth of Stock Testicle

Fig 5—Two days' growth of testicle in plasma plus spleen extract.
Fig 6.—Two days' growth of testicle in old plasma.
Fig 7—Two days' growth of testicle in young plasma



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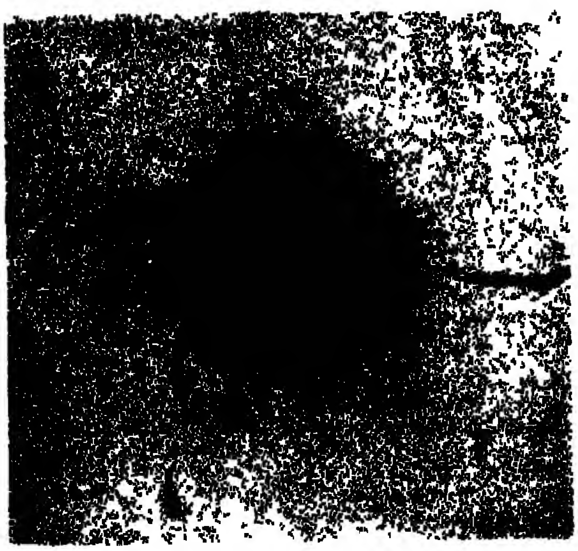

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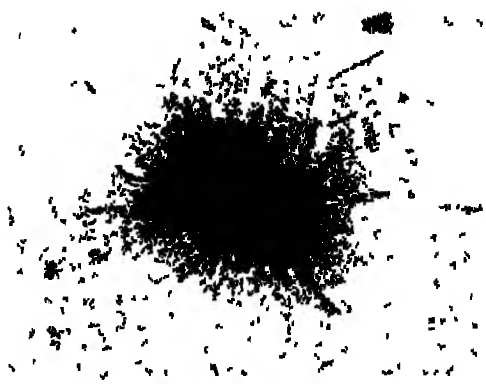

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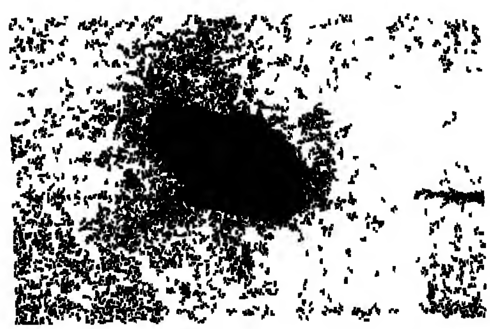

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The Influence of Homodromous and Heterodromous Electric Currents on Transmission of Excitation in Plant and Animal.

By Prof J C Bose, M A D Sc, C S I, C I E, Presidency College, Calcutta

(Communicated by Prof S H Vines, F R S Received June 2, 1914)

I have in a previous paper* described investigations on the conduction of excitation in *Mimosa pudica* It was there shown that the various characteristics of the propagation of excitation in the conducting tissue of the plant are in every way similar to those in the animal nerve Hence it appeared probable that any newly found phenomenon in the one case was likely to lead to the discovery of a similar phenomenon in the other

A problem of great interest which has attracted my attention for several years is the question whether, in a conducting tissue, excitation travels better with or against the direction of an electric current The experimental difficulties presented in the prosecution of this enquiry are very numerous, the results being complicated by the joint effects of the direction of current on conductivity and of the poles on excitability. As regards the latter, the changes of excitability in the animal nerve under electrotonus have been demonstrated by the well-known experiments of Pflüger In a nerve-and-

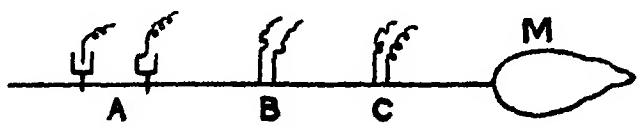


Fig. 1.

muscle preparation, the presence of a pole P is shown to induce a variation of excitability of a neighbouring point S. When P is kathode, the excitability of the point S, near it, is enhanced; stimulation of S, previously ineffective, now becomes effective, and the resulting excitation is transmitted to M,

* Bose, "An Automatic Method for the Investigation of Velocity of Transmission of Excitation in *Mimosa*," 'Phil. Trans.,' B, vol. 204 (1913).

causing response of the muscle Conversely, the application of anode at P causes a depression of excitability of S. Stimulus previously effective now becomes ineffective. In this manner the transmission of excitation may be indirectly modified by the polar variation of excitability of the stimulated point (fig 1*a*)

In the above experiment it will be noted that for inducing a variation of excitability, the tract of nerve SM, along which excitation is transmitted, need have no current passing through it The presence of a given pole is enough to induce a definite variation of excitability in its neighbourhood. For convenience I shall call this the *Inductive action of a pole*

The characteristic variations of excitability induced by polar action are:—

(1) The enhancement of excitability at or near the kathode,

(2) The depression of excitability near the anode.

The boundary between the two polar extensions is reached at a point between the anode and kathode, this point at which the excitability is unaffected is known as the indifference point.

The question whether the inductive action of electric poles affects the rate of conduction has been investigated by von Bezold* and by Rutherford.† Von Bezold found that both descending and ascending currents at A (fig 1*b*) increased the propagation-time between B and C above the normal. Rutherford found on the other hand that the descending current diminished it The results obtained are thus seen to be indefinite as regards the inductive effect of extrapolar current on conduction

Turning from the inductive effect of neighbouring poles, we have the definite object of enquiry Does the direction of an electric current, as such, cause any selective variation in the propagation of excitation? In other words, will a homodromous current, *i.e.* one which flows in the direction of propagation, help or retard transmission of excitation? Will a heterodromous current on the other hand give rise to an opposite effect? The object of this particular enquiry is to determine the *pure* effect of direction of current on conduction of excitation in a tissue through which a current is flowing. We shall call this the *Dynamic* effect of a current on conductivity and distinguish it from the *Inductive* effect.

The experimental difficulties in isolating the pure effect of current on the intensity and rate of propagation of excitation are very great. In the experiment where "the whole polar region is interposed between the exciting electrode and the muscle, the conditions are (very) complex. I have been unable to find evidence of any marked alteration in propagation rate, unless

* von Bezold, 'Elektr. Erreg Nerven u. Muskeln,' Leipzig, 1861.

† Rutherford, 'Journ. Anat. and Physiol.,' London, vol. 2, p. 87 (1868).

the polarising current is intense or of prolonged duration, in which case it is always retarded. The presence of two polar regions, a cathodic accelerating and an anodic retarding, causes the one change to counterbalance the other"* The above would appear to indicate that a current has either no effect or a retarding action on conduction of excitation. These conflicting results are no doubt due to the disturbing influence of the two poles. But this is not the only source of uncertainty in this investigation. Far more serious is the difficulty which arises, as we shall see, from the escape of the induction current employed as the test stimulus. In the course of this paper I shall show how these experimental difficulties have been overcome, and how definite is the characteristic variation of conductivity caused by the directive action of an electric current. The object of my present paper is primarily the demonstration of the selective conductivity induced in the conducting tissue of the plant by the passage of an electric current After giving the results of this enquiry, I next deal with the question whether the various effects observed in the plant have their parallel in the case of the animal also.

Method of Conductivity Balance.—I have previously carried out an electrical method of investigation dealing with the influence of electric current on conductivity The method of Conductivity Balance which I devised for this purpose† was found very suitable. Isolated conducting tissues of certain plants were found to exhibit transmitted effect of excitatory electric change of galvanometric negativity, which at the favourable season of the year was of sufficient intensity to be recorded by a sensitive galvanometer. A long strand of the conducting tissue was taken and two electric connections were made with a galvanometer, a few centimetres from the free ends. Thermal stimulus was applied at the middle, when two excitatory waves with their concomitant electric changes were transmitted outwards By suitably moving the point of application of stimulus nearer or further away from one of the two electric contacts, an exact balance was obtained This was the case when the resultant galvanometer deflection was reduced to zero. If now an electrical current be sent along the length of the conducting tissue, the two excitatory waves sent outwards from the central stimulated point will encounter the electric current in different ways, one of the excitatory waves will travel with and the other against the direction of the current If the power of transmitting excitation is modified by the direction of an electric current, then the magnitudes of transmitted excitations will be different in the two cases, with the result of the upsetting of the conductivity balance. From the

* Gotch, "Polar Excitation of Nerve" in 'Text-Book of Physiology,' edited by Schäfer, 1900, p. 502.

† Bose, "Comparative Electro-physiology" (1907), Longmans, Green and Co.

results of experiments carried out by this method on the effect of feeble current on conductivity, the conclusion was arrived at that *excitation is better conducted against the direction of the current than with it.* In other words the influence of an electric current is to confer a preferential or selective direction of conductivity for excitation, the tissue becoming a better conductor in an electric uphill direction compared with a downhill

The result was so unexpected that I have been long desirous of testing the validity of this conclusion by independent methods of enquiry. In the paper already referred to, I have described an automatic method for recording the velocity of transmission of excitation in *Mimosa* where the excitatory fall of the motile leaf gave a signal for the arrival of the excitation initiated at a distant point In this method the responding leaf is attached to a light lever, the writer being placed at right angles to it The record is taken on a smoked glass plate which during its descent makes an instantaneous electric contact, in consequence of which a stimulating electric shock is applied at a given point E of the petiole (fig. 2). A mark in the recording plate indicates the moment of application of stimulus After a definite interval, the excitation is conducted to the responding pulvinus P, when the excitatory fall of the leaf pulls the writer suddenly to the left. From the curve traced in this manner the time-interval between the application of stimulus and the initiation of response can be found and the normal rate of transmission of excitation through a given length of the conducting tissue deduced. The experiment is then repeated with an electrical current flowing along the petiole with or against the direction of transmission of excitation. The records thus obtained enable us to determine the influence of the direction of the current on the rate of transmission. I shall presently describe the various difficulties which have to be overcome before the method just indicated can be rendered practical.

The scope of the investigation will be best described according to the following plan.

Part I.—Influence of direction of electric current on conduction of excitation in plants.

1. The general method of experiment
2. The effect of feeble heterodromous and homodromous currents on rate of conduction
3. Determination of variation of conductivity by the method of minimal stimulus and response.
4. The after-effects of heterodromous and homodromous currents.
5. Phenomenon of reversal under moderate current.

Part II.—Influence of direction of electric current on conduction of excitation in animal nerve

1 The method of experiment.
2 Variation of velocity of transmission under heterodromous and homodromous currents.
3 Variation in the intensity of transmitted excitation under the action of heterodromous and homodromous currents.
4 After-effects of heterodromous and homodromous currents.
5 Phenomenon of reversal.

Part I—Influence of Direction of Current on Transmission of Excitation in Plant.

1. *The Method of Experiment.*

I may here say a few words of the manner in which the period of transmission can be found from the record given by my resonant recorder, fully described in my previous paper. The writer attached to the recording lever of this instrument is maintained by electromagnetic means in a state of vibration to and fro. The record thus consists of a series of dots made by the tapping writer, which is tuned to vibrate at a definite rate, say 10 times per second This method of intermittent contact not only removes the error due to friction, but also enables time-relations to be deduced from the record In a particular case whose record is given in Curve 1 (fig 3) indirect stimulus of electric shock was applied at a distance of 15 mm from the responding pulvinus There are 15 intervening dots between the moment of application of stimulus and the beginning of response, the time-interval is therefore 1·5 seconds The latent period of the motile pulvinus is obtained from a record of direct stimulation, the average value of this in summer is 0·1 second. Hence the true period of transmission is 1 4 seconds for a distance of 15 mm The velocity determined in this particular case is therefore 10·7 mm. per second.

Disturbance caused by the Leakage of Current.—Employing this method of record, an attempt was made to determine the changes of velocity under the action of heterodromous and homodromous currents. But a serious difficulty encountered at the beginning of the investigation arose from the leakage of the induction current used as the testing stimulus. This will be understood from a concrete example. The record in fig. 3, for example, shows 15 intervening dots between the moment of indirect application of stimulus (at a distance of 15 mm.) and the beginning of response. The recorded time-interval for transmission was thus 1 5 seconds. The latent period of the pulvinus

obtained on direct stimulation was, as stated before, 0·1 second. Repetition of the experiment always gave a time-interval of 1·5 seconds for indirect and 0·1 second for direct stimulation. Now, on completing the circuit of the constant current, which for convenience I shall indicate as the polarising circuit, the time-interval for indirect stimulation was at once reduced to 0·1 second, which is the value of the latent period for direct stimulation. This happened on the mere completion of the polarising circuit, with current reduced even to zero. It is evident that this untoward result was due to the escape of the alternating induction current, which went not merely across the short path from E to E′, but also round by the circuitous path of the polarising circuit. It was the escaping current which caused the direct excitation of the pulvinus. This particular difficulty I was finally able to overcome by interposing the electromagnetic device of a choking coil, which effectively prevented the passage of the alternating induction current into the polarising circuit (fig. 2).

Precaution has to be taken against another source of disturbance, namely, the excitation caused by the sudden commencement or the cessation of the constant current. I have shown elsewhere* that the sudden initiation or cessation of the current induces an excitatory reaction in the plant-tissue similar to that in the animal tissue. This difficulty is removed by the introduction of a sliding potentiometer, which allows the applied electromotive force to be gradually increased from zero to the maximum or decreased from the maximum to zero.

The experimental arrangement is diagrammatically shown in fig. 2. After attaching the petiole to the recording lever, indirect stimulus is applied, generally speaking, at a distance of 15 mm. from the responding pulvinus. Stimulus of electric shock is applied in the usual manner, by means of a sliding induction coil. The intensity of the induction shock is adjusted by gradually changing the distance between the secondary and the primary, till a minimally effective stimulus is found. In the study of the effect of direction of constant current on conductivity, non-polarisable electrodes make suitable electric connections, one with the stem and the other with the tip of a sub-petiole, at a distance from each other of about 95 mm. The point of stimulation and the responding pulvinus are thus situated at a considerable distance from the anode or the kathode, in the indifferent region in which there is no polar variation of excitability. By means of a Pohl's commutator or reverser, the constant current can be maintained either "with" or "against" the direction of transmission of excitation. The

* Bose, 'Plant Response' (1906); 'Irritability of Plants' (1913), Longmans, Green and Co., London.

former, as stated before, will be designated as the homodromous, and the latter as the heterodromous current Electrical connections are so arranged that when the commutator is tilted to the right, the current is homodromous when tilted to the left, heterodromous

For the purpose of convenience I shall call the constant current the

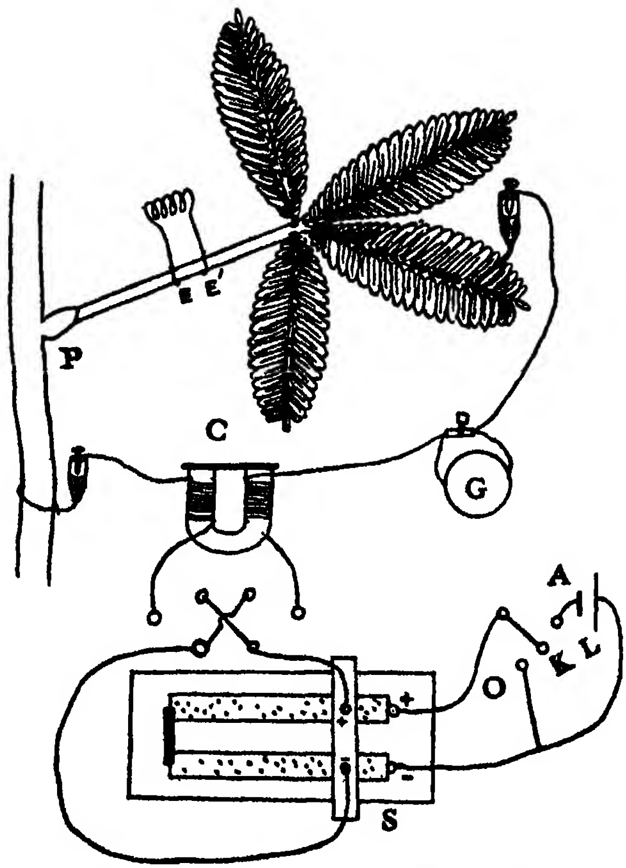


FIG 2.—Complete apparatus for investigation of the variation of conducting power in *Mimosa* A, storage cell, S, potentiometer slide, which, by alternate movement to right or left, continuously increases or decreases the applied E M F ; K, switch key for putting current "on" and "off" without variation of resistance, E, E', electrodes of induction coil for stimulation, C, choking coil, G, microammeter

polarising current, as it will be shown that its effect on conductivity is discriminative or polar, depending on the direction of the current. It should, however, be distinctly understood that under the particular experimental arrangement the possibility of polar variation of excitability at the points of stimulation and region of response is avoided.

The electrical resistance offered by the 95-mm. length of stem and petiole will be from two to three million ohms. The intensity of the constant current flowing through the plant can be read by unplugging the key which short-circuits the microammeter G. The choking coil C prevents the alternating induction current from flowing into the polarising circuit and causing direct stimulation of the pulvinus.

Before describing the experimental results, it is as well to enter briefly into the question of the external indication by which the conducting power may be gauged. Change of conductivity may be expected to give rise to a variation in the rate of propagation or to a variation in the magnitude of the excitatory impulse that is transmitted Thus we have several methods at our disposal for determining the induced variation of conductivity In the first place, the variation of conductivity may be measured by the induced change in the velocity of transmission of excitation In the second place, the transmitted effect of a sub-maximal stimulus will give rise to enhanced or diminished amplitude of mechanical response, depending on the increase or decrease of conductivity brought about by the directive action of the current And, finally, the enhancement or depression of conductivity may be demonstrated by the ineffectively transmitted stimulus becoming effective, or the effectively transmitted stimulus becoming ineffective

Exclusion of the Factor of Excitability.—The object of the enquiry being the pure effect of variation of conductivity, we have to assure ourselves that under the particular conditions of the experiment the complicating factor of polar variation of excitability is eliminated. It is to be remembered that excitatory transmission in *Mimosa* takes place by means of a certain conducting strand of tissue which runs through the stem and the petiole. If a point on the petiole of a given leaf be subjected to strong stimulation, an excitatory impulse will not merely be transmitted across its own pulvinus, but will travel farther along the stem, inducing the fall of other leaves. Conversely, a strong stimulus applied on the stem gives rise to an impulse which passes through the pulvinus to the petiole and thence to the sub-petiole, as evidenced by the successive closure of its leaflets. The main pulvinus may thus be regarded as a contractile indicator of excitation, interposed in the path of the conducting strand which connects the stem with the petiole. In the experiment to be described, the polarising current enters by the tip of the petiole and leaves by the stem, or *vice versâ*, the length of the intrapolar region being 95 mm. The point of application of stimulus on the petiole is 40 mm. from the electrode at the tip of the leaf. The responding pulvinus is also at the same distance from the electrode on the stem. The point of stimulation and region of response are thus at the relatively great distance

of 40 mm. from either the anode or the kathode, and may therefore be regarded as situated in the indifferent region. This is found to be verified in actual experiments.

2 *Effects of Direction of Current on Velocity of Transmission.*

A very convincing method of demonstrating the influence of electric current on conductivity consists in the determination of changes induced in the velocity of transmission by the directive action of the current. For this purpose we have to find out the true time required by the excitation to travel through a given length of the conducting tissue (1) in the absence of the current, (2) against and (3) with the direction of the current. The true time is obtained by subtracting the latent period of the pulvinus from the observed interval between the stimulus and response. Now the latent period may not remain constant, but undergo change under the action of the polarising current. It has been shown that the excitability of the pulvinus does not undergo any change when it is situated in the middle or indifferent region The following results show that under parallel conditions the latent period also remains unaffected —

Table I —Showing the Effect of Polarising Current on the Latent Period

Specimens	I	II.
	sec	sec
Latent period under normal condition	0 10	0·09
" " heterodromous current	0 11	0 10
" " homodromous current	0·09	0·09

The results of experiments with two different specimens given above show that a current applied under the given conditions has practically no effect on the latent period, the slight variation being of the order of one-hundredth part of a second. This is quite negligible when the total period observed for transmission is, as in the following cases, equal to nearly 2 seconds

Induced Changes in the Velocity of Transmission.—Having found that the average value of the latent period in summer is 0·1 second, we next proceed to determine the influence of the direction of current on velocity

Experiment 1.—As a rule, stimulus of induction shock was applied in this and in the following experiments on the petiole at a distance of 15 mm. from the responding pulvinus. The recording writer was tuned to 10 vibrations per second; the space between two succeeding dots, therefore, represents a time-interval of 0·1 second. The middle record, N in fig. 3, is the normal. There are 17 spaces between the application of stimulus and the beginning

of response The total time is therefore 1·7 seconds, and by subtracting from it the latent period of 0·1 second we obtain the true time, 1·6 seconds. The normal velocity is found by dividing the distance 15 mm. by the true interval 1·6 seconds Thus $V = 15/1{\cdot}6 = 9{\cdot}4$ mm. per second We shall next consider the effect of current in modifying the normal velocity. The uppermost record (1) in fig 3 was taken under the action of a heterodromous

Fig 3.—Record showing enhancement of velocity of transmission induced by heterodromous (uppermost curve) and retardation of velocity induced by homodromous (lowest curve) currents N, normal record in the absence of current ← indicates heterodromous, and → homodromous current.

current of the intensity of 1·4 microampères. It will be seen that the time-interval is reduced from 1·7 seconds to 1·4 seconds, making allowance for the latent period, the velocity of transmission under heterodromous current $V_1 = 15/1{\cdot}3 = 11{\cdot}5$ mm. per second In the lowest record (3) we note the effect of homodromous current, the time-interval between stimulus and response being prolonged to 1·95 seconds and the velocity reduced to 8·1 mm. per second The conclusion arrived at from this mechanical mode of investigation is thus identical with that derived from the electric method of conductivity balance referred to previously

That is to say, the passage of a feeble current modifies conductivity for excitation in a selective manner Conductivity is enhanced *against*, and diminished *with* the direction of the current

The minimum current which induces a perceptible change of conductivity varies somewhat in different specimens. The average value of this minimal current in autumn is 1·4 microampères The effect of even a feebler current may be detected by employing a test stimulus which is barely effective.

Table II.—Showing Effects of Heterodromous and Homodromous Currents of Feeble Intensity on Period of Transmission through 15 mm.

Number	Intensity of current in microampères	Period of heterodromous transmission	Period of homodromous transmission
1	1 4	14 tenths of a second	16 tenths of a second
2	1 4	13 " "	15 " "
3	1·6	19 " "	Arrest
4	1 7	12 " "	14 tenths of a second

Having demonstrated the effect of direction of current on the velocity of transmission, I shall next describe other methods by which induced variations of conductivity may be exhibited.

3 *Determination of Variation of Conductivity by Method of Minimal Stimulus and Response.*

In this method we employ a minimal stimulus, the transmitted effect of which under normal conditions gives rise to a feeble response If the passage of a current in a given direction enhances conductivity, then the intensity of transmitted excitation will also be enhanced, the minimal response will tend to become maximal Or excitation which had hitherto been ineffectively transmitted will now become effectively transmitted. Conversely, depression of conductivity will result in a diminution or abolition of response. We may use a single break-shock of sufficient intensity as the test stimulus. It is, however, better to employ the additive effect of a definite number of feeble make-and-break shocks.

We may again employ additive effect of a definite number of induction shocks, the alternating elements of which are exactly equal and opposite. This is secured by causing rapid reversals of the primary current by means of a rotating commutator. The successive induction shocks in the secondary coil can thus be rendered exactly equal and opposite

Experiment 2—Working in this way, it is found that the transmitted excitation becomes effective or enhanced under heterodromous current The homodromous current, on the other hand, diminishes the intensity of excitation or blocks it altogether.

4. *After-Effects of Homodromous and Heterodromous Currents.*

The passage of a current through a conducting tissue in a given direction causes, as we have seen, an enhanced conductivity in an opposite direction We may suppose this to be brought about by a particular molecular arrangement induced by the current, which assisted the propagation of the

excitatory disturbance in a selected direction. On the cessation of this inducing force, there may be a rebound and a temporary reversal of previous molecular arrangement, with concomitant reversal of the conductivity variation. The immediate after-effect of a current flowing in a particular direction on conductivity is likely to be a transient change, the sign of which would be opposite to that of the direct effect The after-effect of a heterodromous current may thus be a temporary depression, that of a homodromous current a temporary enhancement, of conductivity

Experiment 3.—This inference will be found fully justified in the following experiment —The first two responses are normal, after which the heterodromous current gave rise to an enhanced response. The depressing after-effect of a heterodromous current rendered the next response ineffective The following record taken during the passage of the homodromous current exhibited an abolition of response due to induced depression of conductivity. Finally, the after-effect of the homodromous current is seen to be a response larger than the normal (fig. 4). These experiments show

FIG 4 —Direct and after effect of heterodromous and homodromous currents. First two records, N, N, normal. $\downarrow$, enhanced transmission under heterodromous current, $\vee$ arrest of conduction as an after-effect of heterodromous current. Next record $\uparrow$ shows arrest under homodromous current. Last record $\wedge$ shows enhancement of conduction greater than normal, as an after-effect of homodromous current. (Dotted arrow indicates the after-effect on cessation of a given current $\uparrow$ homodromous and $\downarrow$ heterodromous current.)

that the after-effect of cessation of a current in a given direction is a transient conductivity variation, of which the sign is opposite to that induced by the continuation of the current.

5. *Phenomenon of Reversal under Moderate Intensity of Current.*

In studying the effect of increasing intensity of polarising current, the concomitant variations of conductivity appeared at first sight to be very puzzling. The results obtained at different stages were, however, very definite. In the first stage with very feeble current there was no perceptible change of conductivity. At the second stage with increasing current the conductivity variation increased at a rapid rate, and soon attained a maximum. After this, at the third stage, the conductivity change underwent a decline, and then abolition. The effect outwardly resembled that induced at the first stage. There was, however, a difference, for a critical point had now been reached beyond which there was a complete reversal of normal conductivity variation. These different effects will be clearly understood from the following tabular statement:—

Table III.—Conductivity Variations at Different Stages.

	Heterodromous current.	Homodromous current
I stage	No perceptible change	No perceptible change
II "	Enhanced conductivity	Depression of conductivity culminating in a block
III "	Conductivity change reduced to zero at critical point	Conductivity change reduced to zero at critical point
IV "	Reversal diminution of conduction culminating in a block	Enhancement of conduction

I shall now describe a typical experiment which will clearly demonstrate the phenomenon of reversal.

Experiment 4.—In this I was desirous of obtaining with an identical specimen alternate records showing (1) normal effect under feeble current, (2) reversed effect under moderate current, and (3) normal effect once more under the original feeble current. It took two hours to obtain these six records (fig 5) at intervals of 20 minutes The specimen was vigorous, and therefore was little affected by fatigue. The normal enhancement of conductivity under heterodromous current was observed at as low a current-intensity as 1 microampère. In the first of the pair of records (1) we find the interval between stimulus and response under heterodromous current to be 18 tenths of a second, this was prolonged to 21 tenths under homodromous current; the current was next raised to 3 microampères, and we observe in the pair of records (2) the reversal of normal effect by a block of conduction under heterodromous, and transmission under homodromous current; the time-interval in the latter case is 20 tenths of a

second The pair of records (3) was taken once more under the original feeble current of 1 microampère (fig. 5). The plant had by this time become

Fig 5.—Records obtained with an identical specimen showing (1) normal action under feeble current, (2) reversed action under strong current, (3) normal effect once more under feeble current → represents homodromous and ← heterodromous current.

slightly fatigued, the results, however, are similar to those of the first series. We have transmission once more under heterodromous current (the time-interval being 20 tenths instead of 18 tenths of a second), and retardation culminating in block under homodromous current. I give below a tabular statement of results of typical experiments on reversal under moderate current

Table IV.—Reversal under Moderate Intensity of Current.

Number	Intensity of current	Transmission period under heterodromous current	Transmission period under homodromous current.
	microampères		
1	3	Block of transmission	19 tenths of a second.
2	3.5	" "	12 " "
3	4	" "	16 " "
4	4	" "	22 " "
5	4.5	" "	18 " "

The action of a strong current induces a block of conduction under both heterodromous and homodromous currents.

Part II—Influence of Direction of Electric Current on Conduction of Excitation in Animal Nerve.

I shall now take up the question whether an electrical current induced any selective variation of conductivity in the animal nerve, similar to that induced in the conducting tissue of the plant.

1. *The Method of Experiment*

In the experiments which I am about to describe, arrangements were specially made so that (1) the excitation had not to traverse the polar region, and (2) the point of stimulation was at a relatively great distance from either pole. The fulfilment of the latter condition ensured the point of stimulation being placed at the neutral region

In the choice of experimental specimens I was fortunate enough to secure frogs of unusually large size, locally known as "golden frogs" (*Rana tigrina*) A preparation was made of the spine, the attached nerve, the muscle and the tendon The polarising electrodes were applied at the extreme ends, on the spine and on the tendon (fig 6) The following are the measurements, in a typical case, of the different parts of the preparation

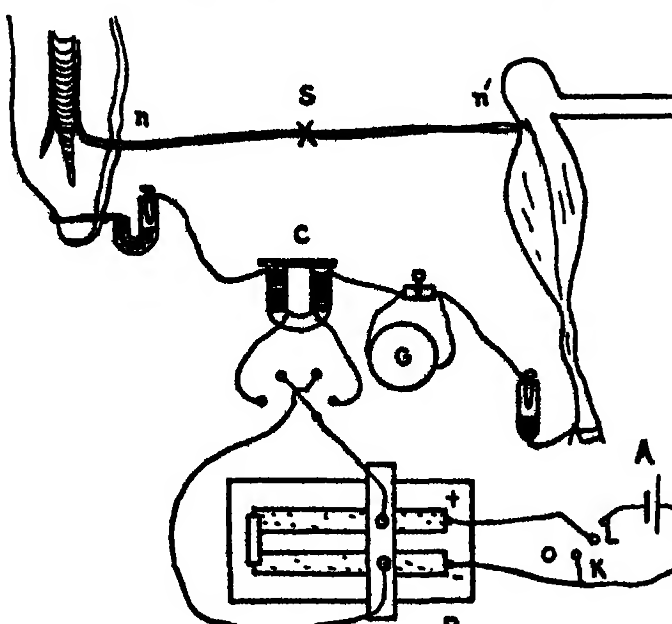


Fig. 6.—Experimental arrangement for study of variation of conductivity of nerve by the directive action of an electric current. *n n'*, nerve ; S, point of application of stimulus in the middle or indifferent region

Length of spine between the electrode and the nerve = 40 mm ; length of nerve = 90 mm , length of muscle = 50 mm , length of tendon = 30 mm. Stimulus is applied in all cases on the nerve, midway between the two electrodes, this point being at a minimum distance of 100 mm. from either electrode. The point of stimulation is, therefore, situated at an indifferent region

Great precautions have to be taken to guard against the leakage of current. The general arrangement for the experiment on animal nerve is similar to that employed for the corresponding investigations on the plant The choking coil is used to prevent the stimulating induction current from getting round the polarising circuit The specimen is held on an ebonite support, and every part of the apparatus insulated with the utmost care

2 *Variation of Velocity of Transmission*

In the case of the conducting tissue of the plant a very striking proof of the influence of the direction of current on conductivity was afforded by the induced variation of velocity of transmission Equally striking is the result which I have obtained with the nerve of the frog

Experiment 5 —The experiments described below were carried out during the cold weather The following records (fig 7), obtained by means of the pendulum myograph, exhibit the effect of the direction of current on the

FIG 7 —Effect of heterodromous and homodromous current in inducing variation in velocity of transmission through nerve. N, normal record ; upper record shows enhancement and lower record retardation of velocity of transmission under heterodromous and homodromous currents respectively.

period of transmission through a given length of nerve. The latent period of muscle being constant, the variations in the records exhibit changed rates of conduction. The middle record is the normal, in the absence of any current

The upper record, denoted by the left-handed arrow, shows the action of a heterodromous current in shortening the period of transmission and thus enhancing the velocity above the normal rate The lower record, denoted by the right-handed arrow, exhibits the effect of a homodromous current in retarding the velocity below the normal rate I find that a very feeble heterodromous current is enough to induce a considerable increase of velocity, which soon reaches a limit. For inducing retardation of velocity, a relatively strong homodromous current is necessary. I give below a table showing the results of several experiments.

Table V.—Effect of Heterodromous and Homodromous Currents of Feeble Intensity on Velocity of Transmission.

Specimen	Intensity of heterodromous current	Acceleration above normal	Intensity of homodromous current	Retardation below normal
	microampère	per cent	microampères	per cent
1	0 35	16	1	20
2	0 7	13	1 5	10
3	0·8	18	2·0	14
4	0·8	11	2·0	13
5	1·0	18	2·5	12
6	1 5	15	3·0	40

3. *Variation of Intensity of Transmitted Excitation under Heterodromous and Homodromous Currents.*

In the next method of investigation, the induced variation of intensity of transmitted excitation is inferred from the varying amplitude of response of the terminal muscle Testing stimulus of sub-maximal intensity is applied at the middle of the nerve, where the polarising current induces no variation of excitability Stimulation is effected either by a single break-shock or by the summated effects of a definite number of equi-alternating shocks, or by chemical stimulation.

Experiment 6—Under the action of feeble heterodromous current the transmitted excitation was always enhanced, whatever be the form of stimulation Homodromous current on the other hand inhibited or blocked excitation (figs. 8, 9)

Complication due to Variation of Excitability of Muscle—In experiments with the plant, there was the unusual advantage in having both the point of stimulation and the responding motile organ in the middle or indifferent region. Unfortunately this ideally perfect condition cannot be secured in experiments with the nerve-and-muscle preparation of the frog It is true

that the point of stimulation in this case is chosen to lie on the nerve at the middle or indifferent region. But the responding muscle is at one end, not very distant from the electrode applied on the tendon. It is, therefore,

Fig 8.—Ineffectively transmitted salt-tetanus becoming effective under heterodromous current

Fig 9—Direct and after-effect of homodromous current. Transmitted excitation (salt-tetanus) arrested under homodromous current, on cessation of current there is a transient enhancement above the normal

necessary to find out by separate experiments any variation of excitability that might be induced in the muscle by the proximity of either the anode or the kathode, and make allowance for such variation in interpreting the results obtained from investigations on variation of conductivity

In the experimental arrangement employed, the heterodromous current is obtained by making the electrode on the spine kathode and that on the tendon anode The depressing influence of the anode in this case may be expected to lower, to a certain extent, the normal excitability of the responding muscle. Conversely, with homodromous current, the tendon is made the kathode and under its influence the muscle might have its excitability raised above

the normal. These anticipations are fully supported by results of experiments. Sub-maximal stimulus of equi-alternating induction shock was directly applied to the muscle and records taken of (1) response under normal condition without any current, (2) response under heterodromous current, the tendon being the anode, and (3) response under homodromous current, the tendon being now made the kathode It was thus found that under heterodromous current the excitability of the muscle was depressed, and under homodromous current the excitability was enhanced.

The effect of current on response to direct stimulation is thus opposite to that on response to transmitted excitation, as will be seen in the following Table

Table VI —Influence of Direction of Current on Direct and Transmitted Effects of Stimulation.

Direction of current.	Transmitted excitation	Direct stimulation
Heterodromous current	Enhanced response	Depressed response
Homodromous current	Depressed response	Enhanced response

The passage of a current, therefore, induces opposing effects on the conductivity of the nerve and the excitability of the muscle, the resulting response being due to their differential actions. Under heterodromous current a more intense excitation is transmitted along the nerve, on account of induced enhancement of conductivity. But this intense excitation finds the responding muscle in a state of depressed excitability In spite of this the resulting response is enhanced (fig. 8) The enhancement of conduction under heterodromous current is, in reality, much greater than is indicated in the record. Similarly under homodromous current the depression of conduction in the nerve may be so great as to cause even an abolition of response (fig. 9), in spite of the enhanced excitability of the muscle. The actual effects of current on conductivity are, thus, far in excess of what are indicated in the records.

The two factors, namely, the induced variation of the conductivity of the nerve and the excitability of the muscle, being antagonistic, certain effects may be predicted when the relative values of the two are changed in definite ways. Let us first consider the effect of diminishing the factor of conductivity of nerve to zero, by bringing the stimulator near the muscle, this being tantamount to direct stimulation. The result is seen in the third column of the Table given above We may next increase the value of the conductivity factor

by increasing the length of the conducting path, *i.e.* by taking greater length of nerve for transmission of excitation. The result is seen in the second column of Table VII. It will now be understood how, by shortening the length of nerve, the normal effect may undergo a reversal. I shall in the following Table denote the change of conductivity of nerve by C_n, that of the excitability of muscle, by E_m.

Table VII.—Reversal of Normal Effect by Shortening the Length of Nerve.

Length of nerve	Direction of Current	Conductivity of nerve *versus* excitability of muscle	Resulting response
1. Long	Heterodromous Homodromous	Enhanced C_n > Depressed E_m Depressed C_n > Enhanced E_m	Enhanced response Depressed response
2. Short	Heterodromous Homodromous	Enhanced C_n < Depressed E_m Depressed C_n < Enhanced E_m	Depressed response Enhanced response

I shall now give experimental verification of the truth of the inferences that have been outlined above.

Experiment 7.—We have seen that, when the nerve is stimulated in the middle or indifferent region, the transmitted effect is normal. From the above we see that this normal effect will persist, as long as the nerve-tract is of sufficient length, and that the effect will undergo an apparent reversal when it is very much shortened. This is fully borne out by results of numerous experiments. For example, the length of nerve in a preparation was 90 mm. When stimulus was applied near the spine (length of transmission = 90 mm.), the transmitted effect was found to be normal, *i.e.* enhanced response under heterodromous, and depressed response under homodromous current. The transmitted effect remained normal as the stimulator was gradually moved towards the muscle, thus reducing the length of transmission. A critical length was now found below which the effects underwent a reversal. This was the case when the length of the nerve was reduced to 15 mm., the reversed effects being an enhanced response under homodromous, and a depressed response under heterodromous current. These are due, as explained before, to the induced variation of excitability of muscle, which now became the predominant factor. The very great influence exerted by the direction of current on conductivity of nerve is forcibly brought to our mind by the fact that under normal conditions it completely overpowers the opposing effect of change of excitability in the muscle.

4. *After-Effects of Heterodromous and Homodromous Currents*

On the cessation of a current there is induced in the plant-tissue a transient conductivity change of opposite sign to that induced by the direct current (*cf* Experiment 3) The same I find to be the case as regards the after-effect of current on conductivity change in animal nerve Of this I only give a typical experiment of the direct and after-effect of homodromous current on salt-tetanus

Experiment 8.—In this experiment sufficient length of time was allowed to elapse after the application of the salt on the nerve, so that the muscle, in response to the transmitted excitation, exhibited an incomplete tetanus. The homodromous current was next applied, with the result of inducing a complete block of conduction, with the concomitant disappearance of tetanus (fig 10)

FIG 10—Normal transmitted salt-tetanus without current at 0 Enhancement under heterodromous current of 3 microampères Reversal at 10 microampères

The homodromous current was gradually reduced to zero by the appropriate movement of the potentiometer slide The after-effect of homodromous current is now seen in the transient enhancement of transmitted excitation, which lasted for nearly 40 seconds. After this the normal conductivity was restored Repetition of the experiment gave similar results.

5 *Phenomenon of Reversal*

In experiments with *Mimosa* it was shown that an increase of polarising current above the critical value gave rise to a reversal of the normal conductivity variation (*cf* Experiment 4) Even in this matter of reversal I find a very remarkable parallelism between the reactions of the conducting tissue of the plant and the nerve of the animal. The reversal was obtained

both with heterodromous and homodromous currents, the testing stimulus being either electrical or chemical

Reversal under Increased Heterodromous Current

Experiment 9.—In this the transmitted excitation due to the application of salt, gave rise to an incomplete tetanus of moderate intensity. After securing this condition, heterodromous current was applied and continuously increased. It will be seen (fig 10) that a great enhancement of conduction took place when the current attained a value of 3 microampères A reversal was, however, induced as soon as the current reached a value of 10 microampères, and we observe a complete cessation of normal incomplete tetanus From this we see that under reversal the conductivity is depressed below the normal

Table VIII.—Showing Normal and Reversed Effects under Heterodromous Current

No	Intensity of current for normal enhancement of conductivity.	Intensity of current for reversed effect of depression of conductivity
	microampères	microampères
1	1·5	6
2	1 5	8
3	2	10
4	2	10
5	3	10
6	3	10 5
7	3	11
8	3	12

Reversal under Increased Homodromous Current

Experiment 10—On the appearance of incomplete tetanus T brought on by the application of salt at the middle of the nerve, homodromous current was continuously increased from zero to 10 microampères and then gradually brought back to zero This was accomplished, as stated before, simply by the forward and backward movement of the potentiometer slide The resulting record taken on the revolving drum shows the cycle of effects. It is seen that the conduction in this case is arrested as soon as the polarising current attains a value of 2 microampères; at 10 microampères there is induced a reversal with an enhanced conductivity above the normal. Under a continuous diminution of current there is an arrest of conduction once more at 2 microampères, and restoration of normal conduction at zero intensity of current. A continuous increase of current gives

rise once more to an arrest of conduction at 2 microampères and a reversal at 10 microampères. It is a curious fact that the reversal under heterodromous and homodromous currents takes place, generally speaking, at the same intensity, namely, 10 microampères

Before passing under review the characteristic results obtained under varying conditions of the experiment, I shall discuss briefly the question whether it is possible to explain the observed results merely by considering the induced variation of excitability as the sole cause. We shall take, then, the simple case of arrest of conduction by homodromous current, I find that the arrest takes place just the same, whether the anodic electrode is placed on the spine or on an adjacent point *n*, on the nerve itself (see fig 6) Discarding from our consideration the possibility of an induced variation of conductivity, we may assume that the arrest was due to the depression of excitability of the stimulated point of nerve on account of the proximity of the anode But the point of stimulation was, in general, placed not near the anode, but in the middle or indifferent region In fact the diminution or arrest of transmitted excitation was observed even in the case where the stimulus was applied at the far end of the nerve, at a distance of about 70 mm from the anode at one end of the nerve, and only 20 mm from the muscle at the other end. Against this it might be urged that under the action of strong currents the anodic depression might extend to a considerable distance

It has, however, been shown that for causing a depression or arrest of transmitted excitation a strong current was not at all necessary, such a depression sometimes taking place under an intensity of current as feeble as 0 3 microampère, the applied E M.F being less than one third of a volt. The difficulty of explaining the observed results by an assumption of induced variation of excitability would thus appear to be insurmountable This difficulty is greatly intensified—indeed borders on the impossible—when we follow the same reasoning as regards the action of increasing intensity of homodromous current beyond the critical point With stronger current, not only will the indifferent point be pushed towards the kathode, but the depression induced by the anode will be so great as to render the stimulated point of the nerve inexcitable. There being no excitation to be transmitted, the response should then undergo an extinction. Instead of this we find that the response shows an actual enhancement, on account of the reversal of the induced variation of conductivity which has already been described. This shows conclusively that the phenomenon we have studied is due not to a variation of excitability, but to that of conductivity

We have seen further that a perfect parallelism exists in the conductivity variation induced in the plant and in the animal by the directive action of the current. No explanation could be regarded as satisfactory which is not applicable to both cases. Now with the plant we are able to arrange the experimental conditions in such a way that the factor of variation of excitability is completely eliminated The various effects described about the plant-tissue are, therefore, due entirely to variation of conductivity The parallel phenomena observed in the case of transmission of excitation in the animal nerve must, therefore, be due to the induced change of conductivity.

I may now briefly recapitulate some of the principal facts established in this paper

The variation of conductivity induced by the directive action of current has been investigated by two different methods —

(1) The method in which the normal speed and its induced variation are automatically recorded,

(2) That in which the variation in the intensity of transmitted excitations is gauged by the varying amplitudes of resulting responses.

The great difficulty arising from leakage of the exciting induction current into the polarising circuit was successfully overcome by the interposition of a choking coil

The following summarises the effects of direction and intensity of an electric current, on transmission of excitation through the conducting tissue of the plant —

The velocity of transmission is found to be enhanced against the direction of a feeble current, and retarded in the direction of the current.

Feeble heterodromous current enhances conductivity, homodromous current, on the other hand, depresses it

Ineffectively transmitted excitation becomes effectively transmitted under heterodromous current. Effectively transmitted excitation, on the other hand, becomes ineffectively transmitted under the action of homodromous current.

The after-effect of a current is a transient conductivity change, the sign of which is opposite to that induced during the passage of current. The after-effect of a heterodromous current is, thus, a transient depression, that of homodromous current a transient enhancement of conductivity

When the intensity of current is gradually increased, the characteristic conductivity variation is also increased, at first slowly, then rapidly. There is a critical intensity of current above which the conductivity variation undergoes a decline, culminating in an actual reversal The effect of heterodromous

current is then a diminution, and that of a homodromous current an enhancement of conductivity

The characteristic variations of conductivity induced in animal nerve by the direction and intensity of current are in every way similar to those induced in the conducting tissue of the plant.

These various effects are demonstrated by the employment of not one but various kinds of testing stimulus Excitation may thus be caused (1) by a single break-induction shock, or (2) a series of equi-alternating tetanising shocks, or (3) by chemical stimulation,

The results that have been given are only typical of a very large number, which invariably supported the characteristic phenomena that have been described. The records given in this paper are photographic reproductions of the original.

Conclusion

The action of an electrical current in inducing variation of conductivity may be enunciated under the following laws, which are equally applicable to the conducting tissue of the plant and the nerve of the animal

1 The passage of a current induces a variation of conductivity, the effect depending on the direction and intensity of current

2. Under feeble intensity, heterodromous current enhances and homodromous current depresses the conduction of excitation.

3. The after-effect of a feeble current is a transient conductivity variation, the sign of which is opposite to that induced during the continuation of current.

4 The normal conductivity variation undergoes a reversal under a strength of current above the critical value The heterodromous current then induces a depression, while the homodromous current induces an enhancement of conductivity

In my 'Researches on Irritability of Plants' I have shown how intimately connected are the various physiological reactions in the plant and in the animal. And I ventured to predict that the recognition of this unity of response in plant and animal will lead to further discoveries in physiology in general This surmise has been justified, for it was by the study of effect of current on the conducting tissue of the plant that I was led to the discovery of the characteristic effects of the direction of an electric current on the conductivity of the animal nerve.

My research assistants, Messrs. Guruprasanna Das, L M S., and Surendra Chandra Das, M A., rendered me very valuable help in this long investigation.

The Measurement of Arterial Pressure in Man. I.—The Auditory Method.

By Martin Flack, Leonard Hill, F.R.S., and James McQueen

(Received December 3, 1914)

From the Physiological Laboratory, London Hospital Medical College (London Hospital Research Fund), and the Pathological Laboratory, Aberdeen University.)

In a previous communication* we showed that when an artery, exposed in a living animal, is compressed in a glass compression tube full of water (Ringer's solution), the pulse, distal to the compression, is not obliterated until the pressure of the water is raised just above the systolic pressure of the blood in the artery, whereas when the same artery, placed on bone, wood or glass, is compressed by the bag of Leonard Hill's pocket sphygmometer, or by the armlet of the sphygmometer, so arranged that it does not embrace the surrounding pulsating tissues, the pulse is abolished by pressures under, and even much under, the diastolic pressure of the blood stream.

These facts are correlated with the manner in which the artery is compressed in each case. Enclosed in the compression tube the artery is compressed by the water equally in a circular fashion so that the rise of external pressure, up to the diastolic pressure, has no effect in producing deformation of the artery. Ultimately, when the compression becomes greater than the diastolic pressure, the artery flattens and changes to the oval shape during diastole It is flattened during systole when the external pressure rises above the systolic pressure. When the carotid artery of the living animal is freed from the surrounding tissues and placed on a watch-glass and compressed by the bag of the pocket sphygmometer, or by the armlet so arranged as not to embrace the pulsing tissues of the neck, the oval deformation sets in at far lower pressures and is complete in relatively thin-walled labile arteries at pressures much under diastolic pressure. Consequently the blood flow is cut down by an external pressure less than diastolic to a mere ineffective trickle of blood, and the pulse is completely damped out If a branch of the carotid artery distal to the bag were connected with a C-spring manometer, the record would show a progressive lowering of both the systolic and the diastolic pressure almost to zero, till with an external pressure much less than the diastolic pressure in the aorta the pulse was damped out and the blood flow became a trickle.

* 'Roy. Soc. Proc.,' B, vol. 87, p. 344 (1914).

Now MacWilliam and Melvin* have studied the behaviour of an excised artery compressed in a tube containing water with external pressures from zero up to diastolic pressure. They find that with pressures, say, from zero up to 50 mm Hg, the distal manometers, systolic and diastolic, give practically unchanged readings. It is only when the external pressures become equal to the diastolic, or above it, that any great alteration in the distal manometer reading occurs. We find that this is so in the case of the carotid of a living animal enclosed in a glass compression tube and compressed by water. On the other hand, in the case of the same artery placed on a watch-glass, a much lower external pressure applied with the bag of the pocket sphygmometer is sufficient to obliterate the pulse.

The question now arises, how far do the conditions of an artery embedded in the tissues and surrounded with the tissue vessels resemble those of an excised artery enclosed in a compression chamber full of water? Were the facts demonstrated by this simple schema applicable, pressures from zero to 50 mm. Hg should have no effect upon the diastolic and systolic pressures in the brachial or radial artery distal to the point of compression

But such a conclusion is contrary to every-day clinical experience. When an armlet is placed on the upper arm and the radial artery is felt at the wrist, and the pressure in the armlet is raised from zero to 50 mm Hg, at some stage, varying under different conditions to be detailed later, the pulse beat at the wrist increases perceptibly in force Accordingly we have here a seeming paradox, augmentation of the pulse produced by an external pressure rising from 0 to 50 mm. Hg—a pressure sufficient to deform an exposed artery lying upon bone and damp out the pulse, but which has no effect on an artery in the simple schema of MacWilliam and Melvin.

The phenomenon in question is obtained with a varying degree of facility in different people In a patient whose systolic pressure was 130 and diastolic 80 mm. Hg, the increase in the force of the pulse beat became perceptible at the wrist when the external pressure in the armlet on the upper arm was raised to 35–40 mm. Hg. After taking exercise for three or four minutes, when the heart had been made to beat violently and the pulse-pressure† range increased in extent, a condition approaching to the findings in aortic disease, an increase in the force of the pulse beat became apparent with 10 mm. of external pressure in the armlet

In a case of aortic disease with a large pulse-pressure range, the increase in the force of the pulse beat became apparent with 5–10 mm. of external pressure in the armlet.

* 'Heart,' vol. 5, p. 153 (1914).

† The difference between the diastolic and systolic pressures.

From a case of aortic disease having an aberrant radial artery we have taken tracings, using the Dudgeon sphygmograph with weight extension. The absence of the plethysmograph effect and of disturbance from venæ comites—there are none accompanying the aberrant artery—was thus secured. In the tracing (fig 1) the external pressure in the armlet at the upper arm was, to begin with, 0 mm, then raised to 30 mm. The size of the initial pulse beat was regulated by the weight in the pan of the extension apparatus, some little skill is needed to secure the proper weight to show the phenomenon in a marked fashion.

Fig 1

The tracing demonstrates that there is an increase in the systolic pressure in the radial artery as a result of compressing the upper arm; there is consequently an increase in the pulse-pressure range

Corroboration of these tracings has been obtained by placing one armlet on the calf and another on the thigh of a boy. In normal boys we find the thigh reading to be higher than that of the calf.*

Boy	Systolic pressure (horizontal posture).	
	Thigh	Calf
	mm of Hg.	mm. of Hg
I	165	135
		140 when 60 mm. Hg was maintained in the thigh armlet.
		130 " 0 " " "
II	155	120
		135 " 40—50 mm Hg " "
III	105	80
		90 " " " "

* In the case of two patients we have observed a systolic pressure considerably higher in the calf than in the thigh, *e g.*, 175 thigh, 300 calf, upper part, and 225 mm. Hg lower part of leg These differences of pressure obtained in both legs. It has been suggested to us that the higher reading in the leg may be due to protection of the artery from compression by fasciæ It is difficult to see how this can come about, and we are at present unable to offer an explanation other than one based on the observations detailed in the following paper. The difference was certainly not due to resistance to compression on the part of the arterial wall, for the dorsalis pedis artery in these patients offered no unusual

The measurements of systolic pressure thus show an increase in the calf reading brought about by raising the pressure in the thigh armlet to 40–50 mm Hg.

We find that the increase in the force of the pulse beat in the artery distal to an armlet coincident with the rise of armlet pressure from 0 to 50 mm. is correlated with the appearance of certain sounds audible with a stethoscope (Bowles' tambour form was used) placed at the elbow

An armlet is placed on the upper arm and the stethoscope laid without pressure at the bend of the elbow, on raising the pressure in the armlet above systolic pressure and then lowering it gradually, clear sounds became audible, gradually getting louder, these are followed first by murmurs and then by loud clear sounds. At a certain level, which is taken as the index of the diastolic pressure, the loud clear sounds suffer a sudden diminution in volume and tone, to be succeeded by dull sounds, these may be audible much below diastolic pressure, *e g* 28–35 mm. or so

These dull sounds, we believe, are due to the sudden tension of the artery and its branches produced by the impact of the systolic wave This sets the mass of tissue enclosed in the armlet into vibration, the vibrations becoming audible to the ear as sounds. In the case noted previously these dull sounds appeared when the pressure in the armlet enclosing the upper arm reached 35–40 mm. Hg At precisely the same level the force of the pulse beat could be felt increased while palpating with the fingers the radial artery at the wrist. When the subject took violent exercise the dull sounds became audible when the pressure in the armlet reached 10 mm. Hg, and increased in loudness with successive increments of this pressure

In aortic disease, where the pulse-pressure range is high and the artery with each systole suffers considerable distension, the sounds are audible at the bend of the elbow under ordinary conditions. These sounds are at once increased in volume when the pressure in the armlet is raised 5–10 mm , at precisely the same levels of external pressure the finger feels the pulse grow stronger, and the sphygmographic tracing confirms the sensory impressions from the fingers.

Therefore we conclude that a sound is given forth dependent on the pulse-pressure range of the blood stream which enters the armlet. If that range is high, *e.g.* in aortic disease, the systolic wave is big enough to cause the sounds to be produced under ordinary conditions. If the pressure within the armlet is raised 5–10–20–30–40 the systolic wave is reinforced by the

resistance to compression by the bag of the pocket sphygmometer. Further, it is difficult to suppose that the arteries in either leg should offer the same local resistance to compression and a resistance wholly different to that of arteries in other parts of the body.

increased tension of the arteries under the armlet and the sound becomes louder.

The bigger the pulse wave passing to the armlet, the ampler will swing the vessels under the armlet and the less the compression required to increase the force of the pulse wave in the radial artery at the wrist, and produce the dull sounds We believe these sounds are produced by the impact of the systolic wave vibrating the tense artery and its branches, big and small. Consequently they should be independent of the blood flow. An artery ligated beats up to the point where the ligature is applied Normally we feel pulses by closing the artery with the ball of the finger or thumb and feeling the impact of the systolic wave on the tip of the finger or thumb To deform the artery with the finger a lower pressure than the diastolic pressure suffices. The finger deforms it just as the bag of the sphygmometer deforms an artery placed on bone.

It is possible to arrange an armlet on the upper arm and a second armlet immediately below the tambour at the bend of the elbow Suppose the pressure is raised in the armlet to 30–40 mm Hg, sufficient to call forth the dull sounds, and that then the armlet pressure below the bend of the elbow is raised far above the ascertained systolic pressure of the blood. This stops all effective flow in the artery at the bend of the elbow, the subsidiary branches of the main arterial trunk rapidly fill up, as all exit for the blood is blocked in the distal area by the compressing armlet placed below. Yet the dull sounds persist Further, these sounds become more audible as the blood flow is stopped That is exactly what one would expect if the sounds are due to sudden tension The artery and all its branches become tenser above the block. The whole kinetic energy of the pulse spends itself in striking the tense labile artery and its branches The whole mass of tissue under the armlet—permeated with blood vessels—is struck by the pulse.

We find that the phenomenon of an increase in the pulse force in the radial artery at the wrist is often felt best at the first examination of the patient with the sphygmometer The excitement produced by examination increases the force of the heart, and the high crest of the pulse wave reaching the tissues compressed by the armlet becomes reinforced: an increase in the force of the pulse beat is thus felt easily with a low pressure within the armlet As the excitement subsides, and the pulse beat becomes normal in its range, the level of pressure at which the increase is felt becomes higher.

As regards the production of the loud sounds and murmurs heard on passing from diastolic level to systolic level, it is possible to separate the element of sound due to pure tension of the arterial wall and the element of sound which requires a flow of blood.

Suppose one raises the pressure in the armlet on the upper arm to 100 mm. and so develops at the elbow the loud characteristic murmur If one then raise the pressure in another armlet placed below the auscultating tambour, to well above the known systolic pressure in the artery, this murmur disappears but a sound synchronous with each pulse beat appears in its place This is the dull sound due to the sudden tension of the arterial wall, a sound independent altogether of those vibrations which are set up in the arterial wall by that inrush and outrush of the blood which is synchronous with the crest of the systolic wave On lowering the armlet pressure to the level at which the outflow of blood regains sufficient velocity the characteristic murmur returns. We conclude, therefore, that stoppage of the blood flow by the lower armlet while the pressure in the upper armlet ranges up to systolic pressure, cannot prevent the occurrence of sounds, though it leaves their quality changed The sounds due to vibrations set up by the sudden in and outrush of blood disappear, the sounds due to the periodic sudden tension of the arterial walls persist.

As we have said before, in an artery placed on bone or glass and compressed with an armlet (the armlet not embracing pulsing tissues), or with the bag of the pocket sphygmometer, the pulse is obliterated by pressures below diastolic pressure.

By the reinforcement of the pulse in the vessels of the tissues which are enclosed by the armlet or bag, used in the ordinary way, these critical pressures are successfully passed, and the normal process of arterial deformation proceeds at the proper level So accurate systolic blood-pressure measurements, both auditory, tactile, and visual, become possible. On this mechanism—the conserving effect of the tissue vessels on the pulse—depends the accuracy of the auditory method of estimating the diastolic pressure Without this mechanism the diastolic level would be too low. Thus we have found it so when the bag of the pocket sphygmometer, or armlet, used so as not to embrace pulsing tissues (as described in a previous communication, *loc. cit.*) is applied to the aberrant radial. But when the armlet is applied round the arm then the diastolic auditory index, as heard in the aberrant radial, comes much closer to the truth

It has been noted by MacWilliam and Melvin, and others, that sounds can be produced which are audible at the brachial artery at the elbow when finger pressure is applied to the brachial artery in the arm Hill, McQueen, and Flack (*loc. cit.*) have shown that finger pressure applied discretely to any artery, brachial or radial, deforms the artery in a precisely similar manner as does the bag, or armlet (used with the box so as not to embrace pulsing tissues), when applied to the aberrant radial. The pulse is damped out below

diastolic pressure Consequently the sounds produced by finger pressure on the brachial artery, while they somewhat resemble in quality the normal sounds produced by armlet pressure, do not show a perfect similarity to these Thus, suppose we obliterate the brachial artery with the finger, on releasing it gently we hear for a very short period clear sounds followed by murmurs These murmurs are not in our experience followed by clear sounds and then by dull sounds, as is the case when the armlet embraces the upper arm It is obvious that on slightly releasing the occluding pressure the blood flows in jets into the artery, which is relaxed below the seat of compression Hence the first clear tension sounds When the artery becomes oval in shape, the clear sounds are dulled by the murmurs When the artery becomes circular the pulse wave does not suffice to make tense the now patent artery and produce the dull sounds The finger does not obstruct the peripheral outflow and bring into play the reinforcement due to the vessels of the tissues

If we place an armlet distal to the position of the stethoscope, and by the pressure in this armlet obstruct the blood flow, then on compressing the brachial artery with the finger, only clear sounds are heard for a short period as the artery is compressed and released The murmurs vanish, clear sounds take their place, and the range of sounds is short. As we have pointed out, the whole range of sound is dependent on the resonating effect of the vessels in the tissues surrounding the artery This resonating effect is absent when an artery is discretely occluded by the finger so that surrounding tissues are not included.

If an armlet is placed on the upper arm and the external pressure raised till the clear sounds are produced, just below the systolic level, then if the artery be occluded by the finger between the armlet above and the tambour of the stethoscope below, all sounds vanish Supposing we place the tambour under the lower part of the armlet, then compression of the artery immediately distal to the edge of the armlet does not abolish the sounds. They become clearer, because the energy of the pulse spend itself on the mass under the armlet, the obstruction further tightens up the vibrating drum

The sounds are abolished when the finger occludes the artery between the armlet and the tambour, first, because the artery is not distended by the systolic phase of the pulse wave, secondly, because the sounds produced under the armlet are not now conducted by the fluid column of blood in the artery.

Suppose we place the stethoscope under the lower part of the armlet, that is just above the occluding finger, then the sounds are audible just up to the systolic pressure of the blood, *e.g.*, 110 mm. Hg. But if the stethoscope is placed exactly under the upper edge of the armlet, sounds are audible up to,

say, 200 mm Hg in the armlet, a pressure far above the systolic pressure of the blood. It is clear that when an armlet is placed on the upper arm and the pressure raised above systolic pressure, the systolic wave must meet a sudden check at the upper part of the armlet, hence the arteries are here made tense and the sounds produced by their sudden tension are heard It is possible under these conditions (MacWilliam and Melvin* have observed it in one case) that the sound, if exceptionally loud, may be conducted by bone below the armlet Their case was one of aortic regurgitation in a healthy student—a good athlete

Conclusions.

1. In the measurement of arterial pressure by means of the armlet and sphygmometer the auditory method gives clear indices of systolic and diastolic pressure.

The auditory indices are (1) loud throbs heard in the artery below the armlet when the compression is lowered just below the systolic pressure, (2) a sudden diminution in the sounds when the pressure falls just below the diastolic pressure. We find that these indices depend on the pulsatile flow of blood in and out of the part compressed by the armlet, the artery is deformed by the compressive force and its wall swings out and in when the pulse wave strikes the deformed part When the flow of blood into the limb peripheral to the armlet is obstructed the throb is no longer heard, but is replaced by dull sounds caused by the pulse striking the tense arterial wall

2. Dull sounds are heard, under ordinary conditions, when the compression is reduced below the diastolic pressure. Such slight compression gives occasion to the pulse to produce the dull sound, by obstructing the venous outflow, and thus raising the diastolic pressure in the arteries and the tension of their walls.

3 The bigger the systolic wave the less compression is required to make audible the dull sound.

4. The accuracy of the auditory method depends on the conserving effect which the tissue vessels have on the arterial pulse when the arm is compressed.

5. The method cannot, therefore, be used to give accurate measurements in the case of an artery lying on bone and unsupported by tissue vessels such as the aberrant radial or dorsalis pedis

6. Accurate readings can be obtained from these arteries where they lie embedded in tissues, and the reinforcing effect of the tissue vessels comes into play.

* 'Brit. Med. Journ.,' 1914, A, p. 697.

7. Clinicians know that the pulse in the radial artery becomes more forcible when they begin to compress the arm. At the beginning of compression of the arm, the armlet, by obstructing the venous outflow and making tenser the arteries in diastole, improves the conduction of the systolic wave. The pulse in the radial artery, therefore, becomes reinforced. The dull sound and the reinforcement of the pulse are due to the same cause.

8. Evidence has been obtained then, by experiments on man, of the effect of increased tension of the arterial wall (lessened lability) on the conduction of the crest of the systolic wave

The peripheral conditions affect the lability and the pressure readings.

The Measurement of Arterial Pressure in Man. II.—*A Schematic Investigation.*

By Martin Flack, Leonard Hill, F.R.S., and James McQueen

(Received December 3, 1914.)

(From the Physiological Laboratory, London Hospital Medical College (London Hospital Research Fund), and the Pathological Laboratory, Aberdeen University.)

MacWilliam and Melvin* have demonstrated in the case of the excised artery—compressed in their schema—that a compressing force which was not sufficient to obliterate the pulse caused a great fall in the manometer, which they placed distally to the compression tube To cite an example, the entering pressures in the proximal manometers were: systolic 178 mm. Hg, diastolic 118 mm. Hg. A compressing force of 140 mm. Hg caused a great fall in the distal manometers—systolic became 42 mm. Hg, diastolic 22 mm. Hg. We find that the artery, under these conditions, is flattened during diastole, and the inflow during systole is not of sufficient duration to maintain the distal pressure, supposing the resistance to outflow is unchanged. If the resistance to outflow is increased, no such distal fall of pressure occurs.

Their schema differs in essential points from the conditions which pertain to an artery embedded in living tissues and encircled by an armlet. The pressure within the armlet at first does not deform the artery, but expresses blood from, and increases the peripheral resistance in, the mass of tissue it

* 'Heart,' vol. 5, p. 153 (1914).

encloses, by compressing the capillaries and obstructing the peripheral exits It thus converts the compressed area into a resonating mass; the pulse is not damped down in the labile arteries, but strikes the blood which fills to distension not only the main artery, but every patent arteriole throughout the mass, and causes the whole tense mass to vibrate.

Thus we find in the case of the living subject, if the systolic pressure be 115 mm. Hg and the armlet pressure be kept at 110 mm. Hg, the venous pressure in the limb rises distal to the armlet. If another armlet be put below the first, and the pressure raised within this, the pulse at the radial will not be obliterated until the pressure in this armlet reaches 115 mm Hg If the conditions found in the schema of MacWilliam and Melvin held good in the arm, a far lower pressure in this lower armlet would suffice to obliterate the pulse, for in their schema, under similar conditions, the distal manometers show a great diminution in pressure In the case of the arm, as the pressure is raised in the upper armlet the venous outflow becomes obstructed, and the pulse then strikes a mass of blood congested within the vessels which permeate the tissues, no pressure less than systolic in the lower armlet can prevent the vibration of the mass reaching the radial artery. It is true that the pulse felt in the radial becomes feeble as the pressure is raised in the upper armlet to 110 mm., but the pressure in the radial does not sink, because the blood still flows in and cannot escape from the veins. The pulse in the radial is enfeebled by the resistance which arises from the deformation of the brachial artery brought about by a pressure in the upper armlet of 110 mm. Its force is partly spent in the labile artery above this armlet. The range of pulse pressure below the upper armlet is greatly diminished too, because the diastolic pressure is raised owing to the venous obstruction. There is in consequence a much smaller swing, but this swing cannot be stopped until the pressure in the lower armlet is raised to the full systolic pressure, 115 mm. Hg

The facts we have detailed above show that the simple schema, in which an artery is compressed in a chamber full of water, does not represent the conditions which pertain in the arm.

We have attempted to imitate these conditions in the schema represented in fig. 1.

Two glass compression chambers are filled with water and connected with each other and to a compression bottle. In one is placed a piece of human carotid artery, in the other a schematic representation of the tissue vessels. This consists of a condom (thin-walled, wide rubber tube) filled to distension with chopped rubber sponge. The expansion of the condom is limited by

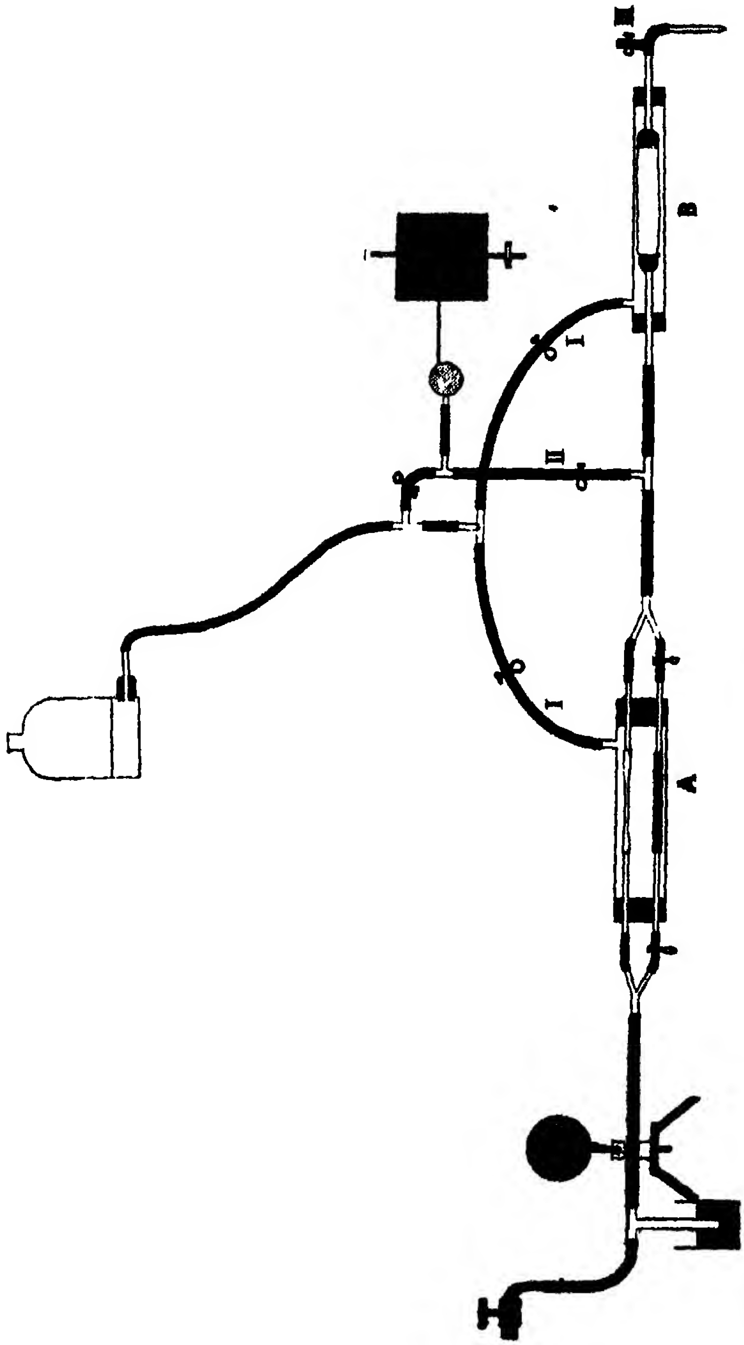


Fig. 1

an external coat of muslin The condom is tied at either end on to a thistle funnel, the tube of each funnel passes through a rubber cork. The corks close the ends of the compression chamber

A pulsatile flow of water is maintained through (A) the artery, (B) the tissue schema. The water finally escapes through a glass nozzle into a pail. The resistance of the tissue schema is such as to give a continuous flow from the nozzle, marked at each systole by a slight pulsatile increase. The pulsatile flow is secured in this wise.

Water flows through the tap through a length of rubber tubing to the schema. Close to the tap a mercury valve is inserted so that the pressure in the tube is kept constant The rubber tube is pulsed rhythmically between two wooden discs (cotton reels), one of which is fixed to the support, and the other to the piston rod itself, of Brodie's respiration pump At each stroke of the pump the tube is compressed and the flow interrupted. The rate of the pulse can be varied. T-pieces are inserted in the schema so that the pulse and pressure can be recorded in turn from (i) one compression chamber or both chambers, (ii) the tube connecting artery and tissue schema, (iii) the outflow nozzle. An alternative pathway is arranged in the compression chamber which contains the artery, so that the flow can be directed either through the artery or through a piece of rubber tube, which acts as a rigid tube Or two pieces of artery, one acting as artery and the other as vein, can be placed in this compression chamber; the flow is then made to pass through (1) the artery, (2) the tissue schema, (3) the vein, and so to the outlet nozzle. Or the flow can be made to go through

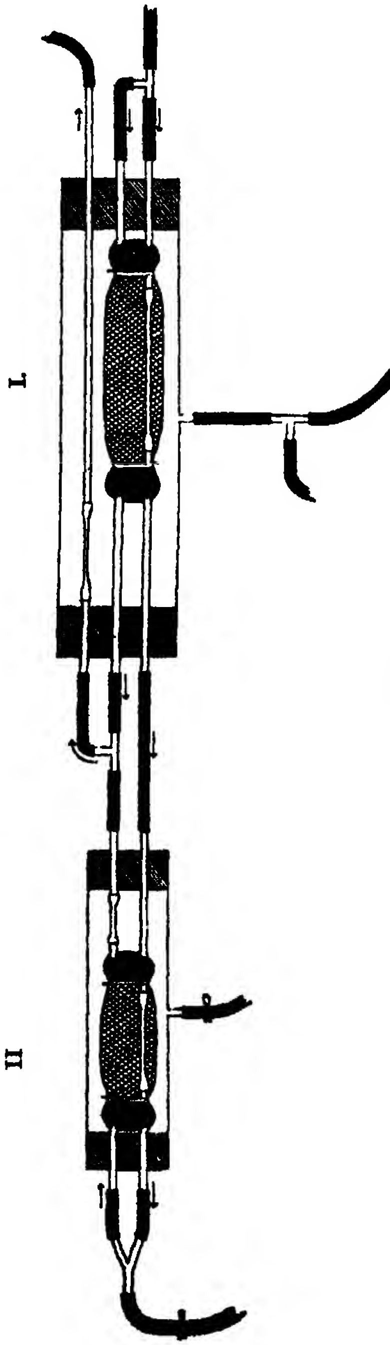


Fig 12.

the artery and vein alone, excluding the tissue schema, or through the artery alone

For the tissue schema we have substituted a human kidney in some of our experiments We finally modified the above schema and made a still closer imitation of the conditions which pertain to the circulation in the arm The artery passes through the tissue schema and is surrounded by it The inflow tube branches and the water flows through both the tissue schema and the artery, the outflow tubes from artery and tissue schema join and pass to another length of artery placed in the same compression chamber, this acts as the vein.

We have used two such complete schemata joined in series in some of our experiments, one representing the upper arm, the other the forearm (fig. 2)

Experiment I

We first observed the effect of circulating water through two lengths of artery—in place of one—both being placed in the same compression chamber The water flowed through (1) the first length of artery, (2) a connecting length of rubber tubing, (3) the second length of artery, and so to the outlet (fig 3).

When the compression chamber was connected with the recording spring

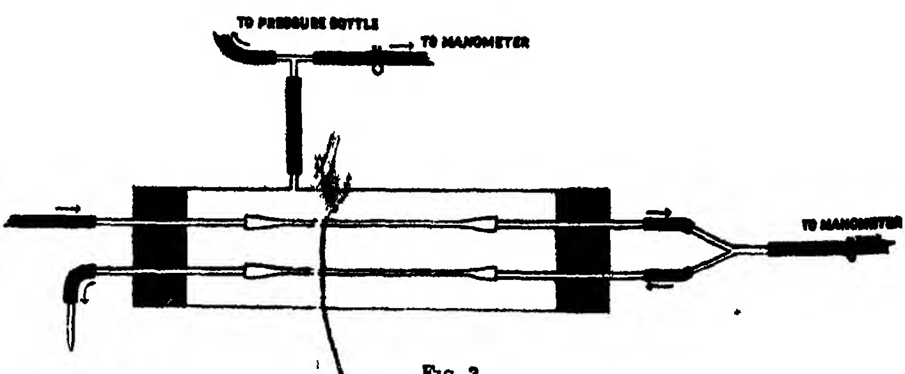
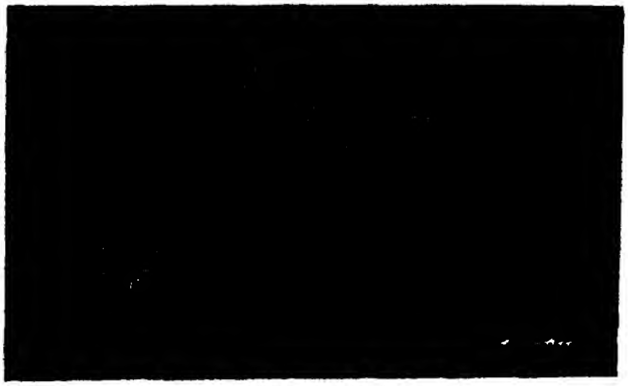


FIG. 3.

manometer the record showed that the maximal pulsation occurred at a lower level when the flow was through two lengths of artery (fig. 4) than when it was through one length (fig. 5).

Owing to the frictional resistance in the length of tube through which the water flowed, the systolic pressure was partly spent in distending the labile first length of artery and in overcoming the frictional resistance during diastole; the second piece of artery, in consequence, had the lower diastolic

pressure, and was thus the first to be deformed and give its maximal swing The first length of artery became taut, owing to the rise of diastolic pressure,



Off On

Fig 4

Off. On.

Fig. 5.

as the second length of artery was flattened. Finally, the first piece of artery was deformed by the increase of compression and gave its maximal swing. The excursion of this maximal swing, owing to the increased diastolic pressure, was smaller, and the pressure at which it occurred higher, than was the case when this length of artery was compressed by itself.

In one such experiment the following were recorded :—

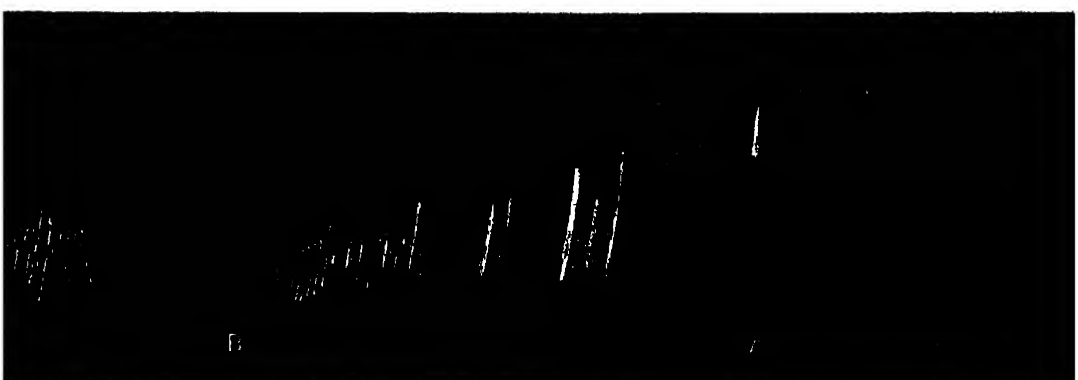
Compression	Deformation	Outflow per minute
One length of artery —		c c
0 cm H_2O	Nil	210
15 "	Nil	
45 "	Begins to flatten visibly in diastole	160
85 "	Flat in systole	Nil
Two lengths of artery—		
0 cm H_2O	Nil	182
15 "	Second length of artery begins to deform	186
45 "	Second length flat in diastole	106
67 "	First length of artery flat in diastole	Drops
85 "	First length of artery flat in systole	Nil

In Experiment I the conditions, of course, are not the same as those which pertain in the arm, for in the arm there is the capillary field with its resistance which precludes the pulse from reaching the veins However, the experiment shows that the behaviour of the artery is notably influenced by the compression of a vessel placed distally to it, and therefore that the study of the compression of a length of artery placed in a simple schema does not suffice to elucidate the compression of the brachial artery in the arm

This conclusion is confirmed by Experiment II.

Experiment II.

We repeated Experiment I, but recorded the pressure in the tube which joined the first and second length of artery. On raising the compression to 5 cm H_2O the second length of artery began to flatten in diastole, the first length became distended in diastole, and the record then showed a rise in pressure A maximal pulse developed as the compression increased.



Off. On. Off On.

FIG 6.

Finally the first length of artery flattened (fig 6, A) On repeating the experiment upon the first length of artery by itself we found it began to flatten and give a maximal pulse at 38 cm H_2O, the pressure then fell in the manometer and reached zero as the compression was increased (fig 6, B)

Experiment III

We varied Experiment I by arranging the outlet in a U-tube containing mercury, so that the resistance to outflow and diastolic pressure increased *pari passu* with the compression (fig. 7). The diastolic pressure being thus

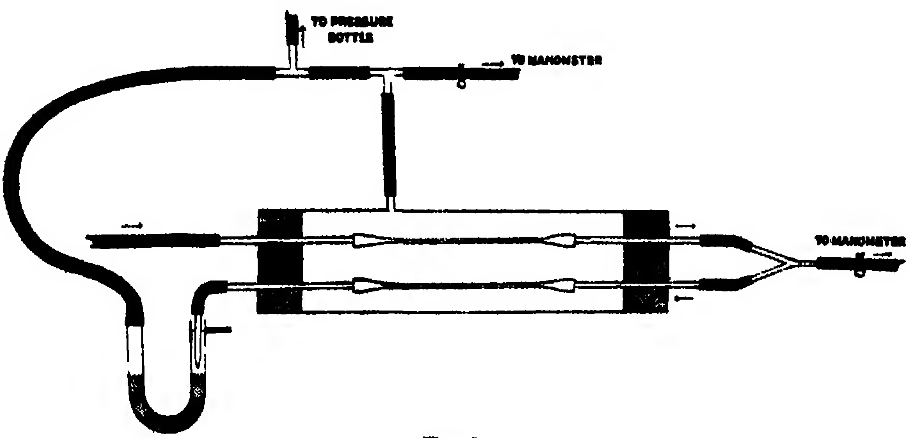


FIG 7

raised *pari passu* with the compression, the maximal pulsation appeared just before the point at which the artery was flattened in systole The result under these conditions was, of course, the same whether one length or two lengths of artery (or artery, tissue schema, and vein) were used Their walls were equally stretched and made more and more rigid by the rising diastolic pressure There was thus little loss of systolic force as the pulse passed along the tubes. On the other hand, the pulse transmitted to the compression chamber and recorded by the manometer became less as the arterial wall became more rigid. When the diastolic pressure which pertained in the schema was over-topped, the length or lengths of artery began to flatten, and a maximal pulse resulted

MacWilliam and Melvin* conclude from their study of the simple schema that the diastolic pressure and maximal pulse do not always coincide. By our experiments we bring into play the effect on the artery of obstructed venous outflow, and find the diastolic pressure and maximal pulse in agreement.

* 'Heart,' vol. 5, p. 153 (1914).

For let us consider the arm when it is compressed. The veins and capillaries under the armlet are first flattened and some are emptied, the pressure rises *pari passu* with the compression in all the remaining patent blood-vessels enclosed by the armlet. This must be so, for their outlet is obstructed In the forearm, peripheral to the armlet, the venous pressure will steadily rise, and the veins become more and more swollen and tense if the compression is maintained just below the arterial pressure Under these conditions the arterial blood can flow into the forearm, so long as capillary fields, hitherto empty, open out and the veins swell, the forearm becoming more and more congested

If a second armlet be put just below the first, and the pressure raised equally in the two armlets, the conditions are made the same as in Experiment II, in so far as the peripheral resistance in the arm is concerned. But, be it noted, as the blood has other arterial pathways open to it in the rest of the body, the diastolic pressure in the arteries enclosed by the upper armlet is not raised nearly up to the systolic pressure. To make the conditions in that the same as in Experiment II, the resistance to outflow in all the arteries would have to increase *pari passu* with the compression of the arm

In the case of the vessels enclosed by the lower armlet under these conditions the diastolic pressure is raised nearly up to the systolic pressure. Now, we have determined experimentally that the reading of systolic pressure taken with an armlet round the calf is raised by placing a second armlet round the thigh and raising the pressure therein to, and keeping it at, say, 50 mm. Hg This correspondingly increases the diastolic pressure in the veins and arteries of the leg, and the arteries, being made more rigid thereby, conserve better the crest of the systolic wave in its passage from thigh to calf. Similarly, the pulse in the radial artery increases in amplitude at first when the compression is raised in an armlet placed round the upper arm, because the compression by obstructing the outflow and making tenser the arteries aids the conduction of the systolic wave.

Experiment IV.

A single length of artery was compressed and the outflow measured. The compression chamber was connected with the manometer When the compression reached 25 cm. H_2O the artery began to flatten. At 34 cm. H_2O the pulse became maximal, and the water then issued in strong pulses; 212 c c flowed out in one minute. At 47 cm H_2O the water issued in shorter pulses, for the artery remained deformed for a longer period during each diastole, the outflow was reduced to 166 c c At 70 cm H_2O the outflow was reduced to feeble spurts synchronous with the systoles, while at 77 cm.

H_2O the outflow ceased to pulse and was reduced to fast drops. The artery then appeared flattened all along its length, but its end proximal to the pump was slightly expanded by each systolic wave At 87 cm. H_2O drops still escaped from the outflow nozzle

Experiments on excised arteries have been undertaken* to test the correctness of the obliteration method of measuring the systolic blood pressure, and the complete cessation of outflow has been taken as the index of obliteration. Wrong conclusions have thus been drawn as to the power of the arterial wall to resist compression The disappearance of the pulse at the distal end must be taken as the index of obliteration, not the absolute cessation of outflow.

Experiment V

The flow was through (1) a length of artery, (2) tissue schema, (3) a second length of artery acting as vein. All these were placed in the same compression chamber, and this connected to the recording manometer (fig 8). The

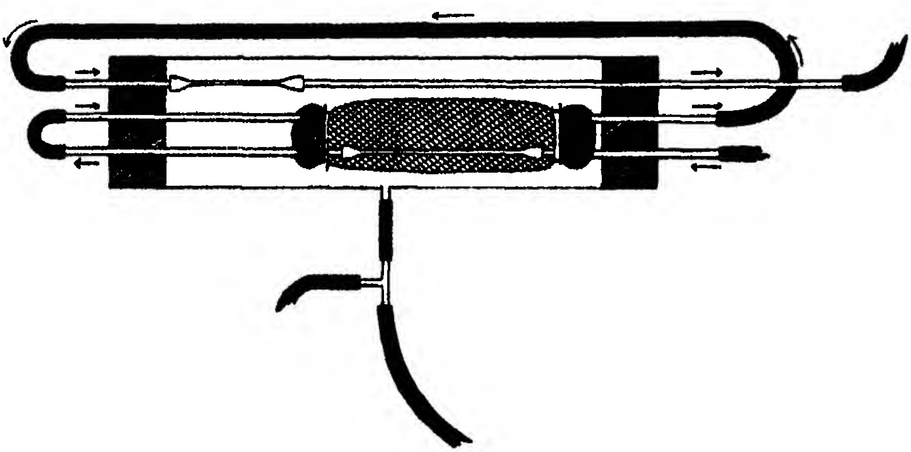


Fig. 8

tissue schema was not tightly packed with chopped sponge, and the pulse travelled through it to the vein.

On compression the vein first flattened and gave a maximal pulsation, while the artery became taut, then the tissue schema shrank The outflow at this period was partly due to the water expelled from it The recorded pulse now became very small as the whole system (artery, tissue schema, vein) was raised up to the diastolic pressure and approximated to a rigid system. Finally, the diastolic pressure was overtopped in the artery, and this gave a maximal pulse (fig. 9).

* Herringham and Womack, 'Brit. Med. Journ.,' 1906, B, p 1614.

Fig 9

The following were the outflows at each stage :—

Compression	Outflow	Compression
cm H_2O	c c per min	
0	146	
5	71	Vein beginning to deform during each diastole
10	68	Maximal pulse of vein
40	33	Tissue schema shrinking Manometer scarcely pulses at all
60	11	Artery beginning to deform during each diastole
65	2	Maximal pulsation of artery

Experiment V elucidates the behaviour of the brain when compressed When the brain is compressed by fluid forced into the subdural cavity the capillaries, venules, etc, similarly shrink, the pressure rises in these vessels, and the whole cerebral vascular system approximates to a rigid system and gives a small cerebral pulse Similarly, when the armlet compresses the arm, part of the blood contained in the tissue vessels is expelled and the remaining patent vessels approximate to a rigid system, in which arterial pressure pertains and through which a diminished flow continues until these are emptied, the artery itself is then flattened, that is, when the systolic pressure is overtopped.

Experiment VI

In Experiment VI the flow was arranged through the artery and the tissue schema placed in separate compression chambers These chambers were connected with each other and the manometer (fig. 1).

A On compression the tissue schema first shrank, then the artery began to flatten and the maximal pulse resulted. On decompression the maximal pulse was more ample and occurred at a lower level than on compression (fig. 10).

Like results were obtained when the tissue schema was replaced by the

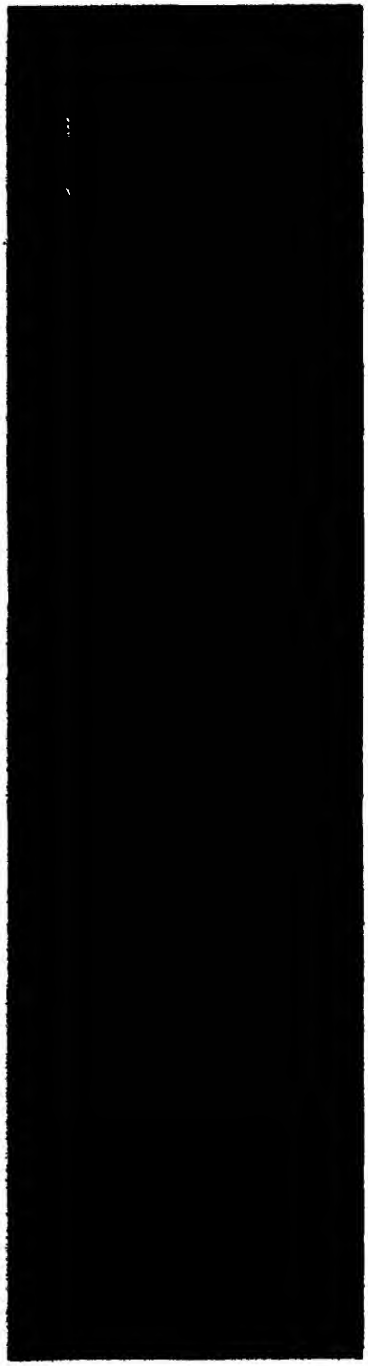


Fig. 10.

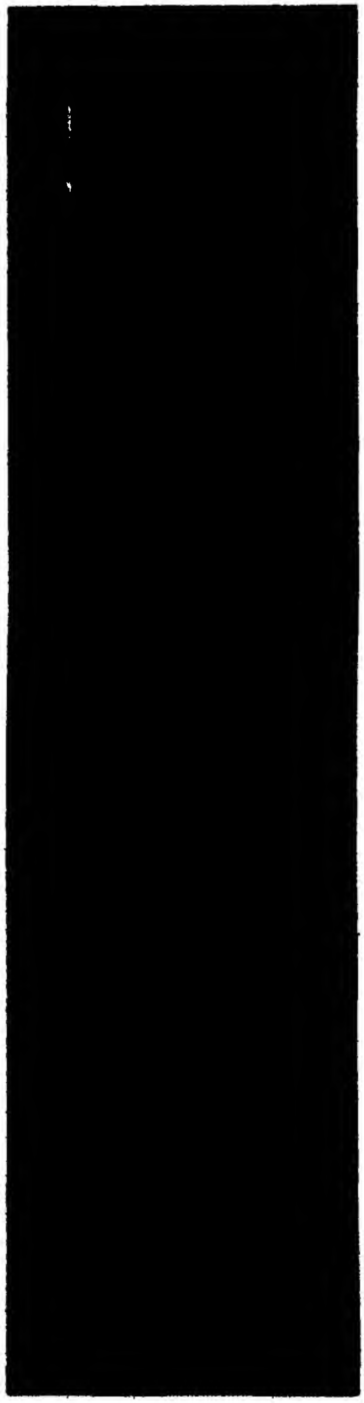


Fig 11

kidney, the length of artery being connected to the renal artery and the renal vein to the outflow.

The compression chambers being connected with each other, a part of the pulsatile force transmitted through the artery to its chamber is conveyed to the chamber of the tissue schema and helps to pulse fluid out of the tissue schema.

When decompression is begun the tissue schema is shrunken, and it takes time to fill out. The outflow is in drops while the expansion is going on. The pulsatile force transmitted through the artery to its chamber is now less spent on the shrunken schema, for this is a more rigid structure. On the other hand, the pulse transmitted directly along the artery to the tissue schema spends part of its force in expanding the shrunken tissue schema. So long as the tissue schema acts as a rigid structure and stores little of the systolic force, the diastolic pressure in the artery will fall to lower level, and, in consequence, the pulsatile swing will be bigger.

The recorded pulse is the summation of that from either chamber, the pulse of the artery, and of the tissue schema.

There is a certain degree of expansion of the tissue schema, which favours the development of a maximal pulse, the stage when the arterial pulse is spent least on, and reinforced most by, the tissue schema.

If the compression and decompression be done in stages, and time be given between each stage for the tissue scheme to shrink or expand, then the maximal pulse occurs at the same height on decompression as on compression.

B The share which the tissue schema takes in the phenomena is shown in fig. 11. The artery was replaced by a rubber tube (rigid). On compression the tissue schema shrank and gave a maximal pulse. On decompression the tissue expanded, and the recorded pulse in this case became smaller because the pulsatile force was spent largely on the expansion of the tissue schema.

Phenomena of the same order happen when the tissues of the arm are compressed by the armlet, and hence arise those differences between compression and decompression readings of systolic pressure which are so often recorded.

Experiment VII.

The flow was through a length of artery and the tissue schema placed in series (fig. 1). Each was in a separate chamber, and these were joined together and to the compression bottle. The tube connecting the artery and tissue schema was joined to the recording manometer.

A. Compression of both artery and tissue schema (fig. 12). The pressure first rose and the pulse amplitude diminished owing to the shrinkage of the

Off Fig 12. On

tissue schema, and the greater resistance thus developed in it, and higher diastolic pressure consequent in the artery. Then a fall of pressure accompanied by maximal pulses resulted owing to the artery flattening during diastole Finally the artery shut up and the pulse ceased to reach the manometer On taking off the compression the pressure and outflow did not return to their previous amounts until the shrunken tissue schema had expanded.

B The compression tube leading to the tissue schema chamber was closed On compressing the artery it began to flatten, and gave maximal swings, and then shut up. No rise of pressure occurred as in "A," because the tissue schema was not compressed (fig 13).

Off. Fig 13. On.

C. The compression tube leading to the artery chamber was closed (fig. 14). On compressing the tissue schema the pressure rose and the pulse

Off. On.
Fig 14.

amplitude diminished; the tissue schema shrank and became more rigid as the resistance to flow was increased. The artery was not itself compressed in this experiment, but acted as a rigid tube in its closed chamber

Experiment VIII.

The flow was through two lengths of artery joined in series Each length was placed in a separate compression chamber. Tubes connected the two chambers with each other and with the manometer.

A The tube leading from the compression chamber of the vein was closed The artery alone affected the manometer

A C B.

Fig 15

B Both compression chambers were open, both artery and vein affected the manometer

C. The compression chamber of the artery was closed. The vein alone affected the manometer.

When both chambers were open the pulsatile expansion of the artery was transmitted from the arterial to the venous compression chamber, and partly spent itself in pulsing fluid out of the vein. When the venous chamber was closed this no longer occurred, and the vein became a rigid tube and no longer stored the systolic force. The diastolic pressure therefore fell in the artery, and this gave a bigger swing, which acted with undivided force on the recording manometer. The like result was obtained when we replaced the second length of artery by a kidney, and connected the renal vessels, so that the flow went through (1) the length of artery, (2) renal artery, (3) renal capillaries, (4) renal vein The effect was not obtained when we substituted a length of rubber tube (rigid) for the length of artery.

In the case of the brain or other encapsulated organ the arterial pulse transmitted through the substance of the organ helps to pulse blood out of the venous sinuses. In the case of the kidney urine is pumped out of the collecting tubules as well as blood out of the renal veins by each pulsatile expansion of the organ.

Experiment IX

The following was an experiment in which the result is to be explained on the same lines.

The flow was through the artery and the tissue schema placed in series and each in a separate compression chamber These chambers were connected with each other and with the pressure bottle

The tubs connecting the artery with the tissue schema was connected with the manometer The compression was raised until the artery flattened in diastole and the manometer gave maximal swings On closing the tube leading to the compression chamber of the tissue schema the artery flattened to a greater extent and the pulse amplitude became diminished (fig 16) The explanation is as follows —

The tissue schema then became a rigid structure and no longer stored the

FIG 16.

systolic force Therefore the diastolic pressure in the artery diminished and the artery was more effectually closed by the compression during diastole. The systole in its turn was less effectual in driving fluid through the artery. The systolic force was spent more on the lability of the artery, *i.e.*, in opening it out

Like results were obtained when we substituted the kidney for the tissue schema.

When the chamber containing the artery was closed in place of that

FIG. 17

containing the tissue schema, the results were as follows, when the compression was arranged to produce a maximal pulsation. When the chamber was closed at the moment of diastole the artery became fixed in diastole and remained flat, so that the pulse scarcely came through (fig 17, A) Closing the outlet now restored both pressure and pulse, for as the flow was thus completely stopped the artery distended On the other hand, when the chamber was closed at the height of systole the artery was fixed fully open and the pulse came through, but with a diminished diastolic excursion (fig. 17, B)

Experiment X

The artery and vein were in one compression chamber, the kidney in another separate chamber The flow was from artery to kidney to vein. The compression chambers were connected with each other and to the manometer

A. The compression chamber of the artery was closed. On compressing the kidney alone the kidney shrank, and the pulse of the renal arteries became at first more ample owing to lessened lability of the kidney vessels Finally, the outflow almost stopped owing to the resistance in the kidney, and the renal artery, becoming distended, ceased to pulse. On now compressing the artery and vein in their chamber to a like amount the resistance increased in the vein, and the kidney expanded so that the pulse and flow began again. It took a much higher degree of compression applied simultaneously to artery, kidney, and vein to stop the pulse and flow.

B The compression chamber of the kidney was closed. The artery and vein were compressed alone The vein was flattened and the flow ceased, the artery becoming distended, and the systolic force spent in expanding the labile kidney On now compressing the kidney, this organ shrank and became less labile (more rigid), and the pulse and flow began again.

Experiment XI.

The flow is through (1) artery, (2) kidney. Each is placed in a separate compression chamber These chambers are connected together. The tube connecting artery and kidney is joined to the manometer

A. The compression is increased in both chambers The kidney shrinks, the pressure rises, and a maximal pulse of the arteries develops; as the pressure is made greater the flow and pulse cease. On decompression the maximal pulse occurs at a lower level than on compression (fig. 18).

B. The tube leading to the compression chamber of the artery is closed and the artery thus made rigid. The pulse transmitted to the manometer becomes ampler, because it is no longer damped down in its passage through

the labile artery (fig 19) That this is so is shown by the fact that a like result is obtained when a piece of rubber tube (rigid) is substituted for the artery and the compression chamber is left open

Off On

Fig 18.

Off On

Fig 19

When the kidney is compressed the pressure rises and the pulse recorded by the manometer becomes increased still more. The explanation is as follows —

The kidney shrinks and the resistance to the flow through its vessels increases The renal vessels become rigid and no longer store up the systolic pressure. The diastolic pressure falls in consequence, and the full swing of the pulse is thrown upon the manometer

Experiment XII

The flow was through the two complete schemata placed in series, and each in a separate compression chamber (fig 2) The compression chamber of schema II was connected to the manometer

Fig. 20

A The first effect of compressing schema I was to increase the amplitude of the pulse in schema II (fig 20) In this experiment the pulse in schema II was largest when the compression in schema I rose to 40 cm H_2O.

On compressing the arm the pulse in the radial at first increases in amplitude—a fact well known to clinicians The tracing in the previous paper shows this increase in amplitude as recorded in the radial artery Our explanation of this phenomenon is as follows —

The first effect of increasing the compression was flattening of the venous outlet which lay in the first schema. The resistance to outflow was thus increased and, in consequence, the diastolic pressure rose and the arteries and tissue schemata became more rigid, i e less labile Therefore the pulse was less spent on the lability of the artery in the first schema and reached the second schema with greater force On decompression of the first schema the pulse reappeared in the second schema at a lower degree of pressure than that at which it disappeared on compression of the first schema

This was only the case, let it be noted, when the decompression was rapid (fig. 21) As we have said before, the same phenomena is often observed

Off On

Fig 21

when the systolic pressure is measured on man. The reappearance of the pulse generally gives a lower reading than the disappearance On rapid decompression the tissue schema I was suddenly made easily extensile and, in consequence, the diastolic pressure of the artery fell and its lability increased So, too, in the case of the arm when the pressure of the armlet is reduced The full force of the pulse will not reach the second schema (or forearm) until the first schema (or upper arm) is filled and the artery rendered tenser If the forearm is previously emptied of blood by elevation and bandaging before compression, it takes much longer for the pulse to return to its full force on decompression—the bandage being removed before decompression is made. This, too, was the case when the second schema was compressed before the first schema was compressed, and the compression of the second schema removed just before the decompression of the first schema.

Not only the tissue schema of the first but that of the second schema has then to be expanded and rendered tense before the pulse of the second schema becomes restored

Our experiments demonstrate, then, the important influence which the tissue vessels have on the conservation of the pulse in the main arteries

Let us now return for a moment to an experiment we published in a previous paper* On fomenting with hot water the lower arm, and icing the upper arm, we found the radial pulse was obliterated by a pressure which was less when the armlet was applied to the upper arm than when it was applied to the lower arm In the cooled upper arm the tissue vessels and veins were constricted and emptied In the warm lower arm they were flushed and filled The compression applied to the latter at once raised the diastolic pressure in the arteries, and by increasing their tension, *i e* lessening their lability, improved the conduction of the crest of the systolic wave This was not so in the case of the cold upper arm The pulse was not so well conserved by the action of the tissue vessels there, and the brachial artery was thus deformed at a lower pressure We see then how potently the condition of the peripheral circulation may influence the reading of systolic pressure

There is an experiment published by MacWilliam, Kesson and Melvin,† which, it is claimed, refutes all the proofs we have brought forward as to the effect of the lability of arteries on the conduction of the pulse wave. These authors figure two lengths of tubing, one rigid tube, the other artery, connected by a T-piece to the aorta of a cat They connect first one and then the other of these tubes to the manometer, and find the pulse records are the same Therefore, say they, the artery is no more labile than the rigid tube.

We would point out that under their conditions the pulse, as recorded, is affected by the lability of the artery whichever tube is connected to the manometer. To make their experiment adequate they must clip off the artery while recording the pulse from the rigid tube The lability of the artery comes no less into play when it is connected by one end to the rigid tube, as we showed in our previous communication ‡

Conclusion.

1. The simple schema, hitherto used for studying the compression of excised arteries, does not reproduce the conditions which pertain when the arm is compressed

* 'Roy. Soc. Proc.,' B, vol. 87, p. 344 (1914)

† 'Heart,' vol 4, p. 393, Experiment 7 (1913).

‡ 'Roy Soc. Proc.,' B, vol 86, p 365 (1913).

2 A schema has been constructed by us in which artery, tissue vessels, and vein are represented

3. This schema demonstrates some of the principles which govern the circulation in the brain or other encapsulated organ, and the effect of compression upon circulation in such organs

4. Two such schemata arranged in series imitate the conditions which pertain respectively in the arm and forearm, and enable us to demonstrate the effects of compression, and in particular the conserving effect on the pulse of the tissue vessels

5 The schemata demonstrate and elucidate these well-known clinical facts (1) that the pulse may reappear on decompression at a lower pressure than it disappears on compression, (2) that the radial pulse is reinforced when the compressive force applied to the upper arm is below the diastolic pressure

6 The schema demonstrates (1) that the maximal pulse occurs when the diastolic pressure is just overtopped by the compressive force, and is a good index of diastolic pressure, (2) that the diastolic pressure is raised towards the systolic pressure in proportion as the peripheral resistance is increased by compression or obstruction of venous outflow, (3) that this rise of pressure occurs throughout arteries, tissue vessels, and veins, (4) that the pulse is damped down by the lability of the vessel wall, and it is owing to lability that the pulse on decompression returns at a lower pressure level than it disappears on compression.

7. The explanation of the difference which pertains between the arm and leg systolic readings, taken in the horizontal posture, in cases of aortic regurgitation, is to be sought in the difference of the lability of the artery and the conditions of the peripheral circulation The upper limb, as a whole, is more labile than the leg In the normal person the difference of lability is brought out by exercise, which produces a big systolic wave The leg is, so to speak, a tighter drum-head, and responds better to the bigger stroke.

It must always be borne in mind that the support of the column of blood in the artery is not only formed by the tissues of the arterial wall, but also by the surrounding tissues of the whole limb

On the Mechanism of the Cardiac Valves a Preliminary Communication

By A F. STANLEY KENT, M A Oxon., Henry Overton Wills Professor of Physiology in the University of Bristol

(Communicated by Prof. C S. Sherrington, F R S Received February 15, 1915)

(From the Physiological Laboratory of the University of Bristol.)

[PLATE 16]

The account commonly accepted of the manner in which the auriculo-ventricular valves of the mammalian heart are operated relies chiefly upon the action of eddies and currents As the result of recent work it appears possible that a muscular mechanism may be involved also Such a mechanism is referred to in the following communication

It consists of a prolongation downwards of the muscular fibres of the auricular wall in such a manner as to produce a sheet of longitudinally coursing fibres which enter the base of the valve flaps and run a variable distance in their auricular portions *

The general arrangement may best be gathered from the accompanying photographs. These, which have been taken from sections of the hearts of different animals, show that muscular fibres, originating in the auricular wall, sweep down to the valve flaps, which they enter, and become inserted into the connective tissue at some distance from the base.

The passage of this muscle from auricle to valve is uninterrupted, and the tissue found in the valve is similar histologically to that found in the auricle Moreover, the muscle is present in considerable amount, and may even form the greater part of the thickness of the valve It is particularly to be noted that the muscular tissue described as running into the valve arises from and is continuous with the muscular wall of the auricle

A reference to the figures will show that in fig 1, which is taken from a section of the heart of a three weeks old rat, the muscular fibres of the auricle pass without structural change down to the auriculo-ventricular junction, where they come into close relation with the ventricular tissues In the neighbourhood of the mass of connective tissue from which the valve springs the muscle fibres become arranged parallel to the axis of the valve

* I would point out that the muscle described in this paper should not be confused with the interesting sphincter of unstriped muscle, the existence of which at the A-V orifice is mentioned by Prof Gustav Mann in the last edition of Quain's 'Anatomy'

flap, and then run uninterruptedly forward, forming the upper portion of the valve for a distance of about one-half of the total length shown in the section For two-thirds of this distance the thickness of the muscular tissue is considerable At its lower extremity the muscle is thinned out and appears to be inserted into the connective tissue forming the upper layer of the flap

Between the muscle and the underlying fibrous tissue of the valve is a well-marked layer of very loose connective tissue, the presence of which would produce the effect of an insertion of the muscle at a considerable distance from the base of the flap

In fig 2, Plate 16, taken from the heart of an adult cat, the auricular muscle is seen to reach the auriculo-ventricular junction, and then to pass on as a continuous sheet into the valve, reaching in the figure almost to the extremity of the thicker basal part of the flap The muscle fibres are thick and robust even at some distance from the base of the valve

In fig 3, taken from the heart of a child, the auricular muscle, in the neighbourhood of the auriculo-ventricular junction, is seen to be arranged as a series of bundles cut across in the specimen, and as some smaller masses running towards the point of attachment of the base of the valve The muscular fibres enter the valve and run parallel with the axis of the flap They pass down for about a quarter of the length of the flap shown in the specimen, lying in the upper portions of its thickness, and finally ending in relation with the tissues of its upper lamella.

In fig 4, taken from the heart of an adult man, the auricular muscle is seen to approach the auriculo-ventricular junction as two sheets, the inner having a somewhat circular course, the outer having a direction more nearly parallel with the axis of the valve segment

The muscle fibres come to an end in a mass of connective tissue forming the upper part of the segment, the actual point of insertion being at some distance from the point of attachment of the valve

Thus in all the four photographs reproduced the same essential points of structure are shown The auricular muscle passes in considerable mass into the basal portion of the auriculo-ventricular valve, it takes up a position in the auricular part of the segments, and it finally ends by becoming inserted into the fibrous connective tissue of the valve substance As might have been anticipated both mitral and tricuspid valves show the structure described

Muscle in the situation described may well have an important function in connection with the closure of the auriculo-ventricular valves. In accounting for the closure of these valves, authors have commonly relied upon the floating

up of the segments through the action of eddies, and that eddies can actually bring about an approximation of the valve flaps is indicated by an experiment of Baumgarten,* quoted by Sherrington The quotation is as follows —

"If the arterial openings of the excised heart be blocked, and through the auricles a momentary rush of water under about twelve inches pressure be allowed to play into the auriculo-ventricular orifices, the valve flaps rise into the orifice, and come together sufficiently firmly to allow of the inversion of the heart without the escape of a drop of its contents"

Thus eddies or currents may undoubtedly bring about the preliminary approximation of the valve flaps under certain circumstances

The course of events in the normal heart is probably somewhat as follows —

Immediately before the moment at which the valve is timed to close, its flaps are being acted upon by certain forces tending in different directions One of these forces is the stream of blood driven from the auricle, which presses the flaps outwards Another is the eddy behind them, between them and the ventricular wall, which presses them inwards.

To this latter must now be added the effect of the muscular slips described as existing in the bases of the valves, which tend to raise the flaps away from the ventricular wall, and towards the position of closure The actual position of the flaps at any given moment will be determined by the combined effect of these forces.

As the stream of blood driven from the auricle weakens towards the close of auricular systole, the retrovalvular eddy, though possibly also somewhat weakened on account of the lessened stream of blood, will have less to antagonise it. It will therefore become relatively more effective With regard to the muscular action, the contraction of the auricle will have commenced to die away in the upper parts It will still be present in full force in the lower parts of the auricular wall, and also in the slips of muscle entering the valves. That is to say, the muscular contraction will die away and be replaced by a condition of relaxation latest in this situation.

As a result, the flaps will continue to be drawn up by the muscular slips quite to the end of auricular systole, and this will not only have a direct effect in closing the valve at exactly the appropriate instant, but will also ensure free play to the retrovalvular eddy up to the time when the valve closure begins to be finally accomplished.

At the end of auricular systole, therefore, the forces tending to keep the flaps apart will have become weakened, the forces tending to approximate

* Baumgarten, 'Archiv f Anat. und Physiol.,' 1843, p 464. Quoted in Clifford Allbutt's 'System of Medicine,' vol 6 (1909). By C. S. Sherrington Revised by James Mackenzie.

them will still be active. The result is that closure finally occurs, and is rendered absolute by the rapidly increasing pressure of blood in the ventricle which now commences to contract

The function of the muscular slips now described may perhaps be regarded as a double one, (*a*) to keep the flaps away from the heart walls, and thus to ensure the provision of an adequate space between the flap and the ventricular wall for the full development of the retrovalvular eddy, and (*b*) to afford by its contraction actual mechanical assistance to the raising of the flaps into the position of final closure

It is obvious that the anatomical arrangement is admirably adapted to the carrying out of these functions Placed at the base of the auricle and deriving its stimulus thence, the muscle in the valves will contract, and will relax, last of all the auricular tissue, and thus ensure, not only that the mechanical assistance referred to under (*b*) shall become available at the proper moment, but also that the work of the eddy shall be assisted up to the very moment of final closure of the valve.

There are other points of interest connected with the presence of muscular tissue in the segments of the auriculo-ventricular valves which it is not proposed to deal with in the present communication

I wish to record my indebtedness to Mr. R. B. Britton for permission to make use of the specimen from which fig 1 was prepared.

The above research has been assisted by grants from the Government Grant Committee of the Royal Society, from the Research Fund of the University Colston Society, and from the British Association for the Advancement of Science

DESCRIPTION OF FIGURES.

Fig 1—Heart 141204 (Britton) Rat, 3 weeks old. Slide 53, Section 3. × 29. Tricuspid Valve

The auricular muscle is seen to sweep uninterruptedly into the basal part of the valve flap, of which it forms the upper portion, it is inserted into the connective tissue substance at the junction of the basal and middle thirds of the flap as seen in the figure

Fig. 2.—Heart 130614. Slide 252, Section 2 Cat, adult. Tricuspid Valve × 29

The auricular muscle passes without change into the valve, of the thickness of which it forms a considerable part The muscle fibres are situated towards the auricular surface of the flap, lying beneath the endocardium.

A contraction of this muscle would lead to a powerful raising of the valve segment

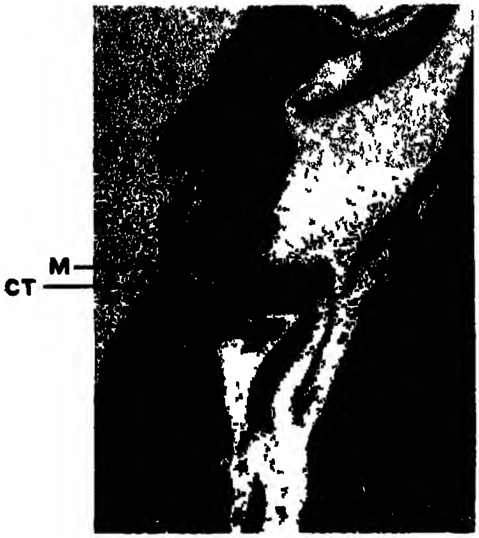


Fig 1

Fig 2

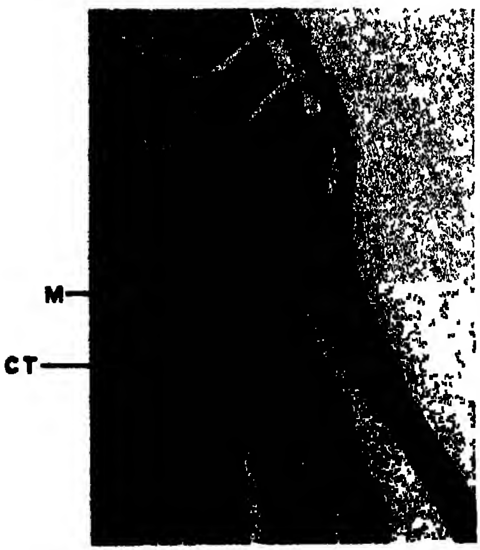


Fig 3.

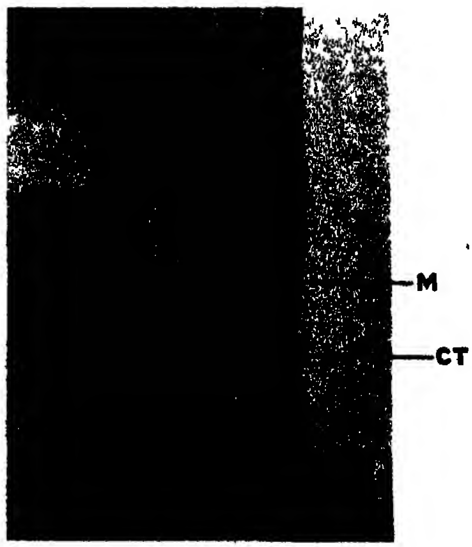


Fig 4.

Fig. 3 —Heart 130522 A. Child, 3 days Slide 293 Tricuspid Valve × 29

The auricular muscle passes into the base of the valve, of which it forms about one-half the thickness It passes for some distance along beneath the auricular endocardium, and is finally inserted into the connective tissue of the valve

Fig 4 —Heart 120214 C Man, adult Series G, Slide 132 Mitral Valve × 7 5

In this specimen the auricular muscle runs a short distance only into the base of the valve Its contraction would, however, be quite efficient in raising the flap, owing to the relative positions of the muscle and the point of attachment of the base of the valve

In all the figures M indicates muscle, CT indicates connective tissue

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The Effect of Functional Activity upon the Metabolism, Blood-flow, and Exudation in Organs.

By J. Barcroft, F R S, and Toyojiro Kato

(Received February 18, 1915)

1 *Striated Muscle*

The animals used were dogs A C E mixture used at first, urethane throughout. Two preparations have been used (*a*) the gastrocnemius preparation as described by Verzár [1], (*b*) the anterior belly of the diagastric In each of four experiments the veins were dissected out which lead from the organ to some adjacent vein of considerable size—the femoral or the jugular All the other confluents to this vein were tied off, and a pipette inserted through one of these into the great vein. This was clamped proximally, and the blood from the muscle, and it only, was thus secured

By measuring its rate of flow, and by comparing it with arterial blood in respect (1) of its oxygen content, and (2) its hæmoglobin value [2], data were obtained of (*a*) the oxygen used by the muscle [3], (*b*) the rate of flow, and (*c*) the exudation [4] of fluid from the blood vessels

In two experiments the nerve was cut, in two it was not The following data were furnished by an experiment on the gastrocnemius, in which this nerve was cut two hours before its stimulation. It was stimulated by a faradic current, of a duration of about 0·2 second, every second for 15 minutes.

Time	Rate of blood flow in c c per min	Oxygen per grm. per min.	Exudation in c.c. per min.
Before commencement of stimulation—			
10 min.	4 6	0 ·0052	0 ·05
During stimulation, calculated from commencement—			
3 min	6 ·7	0 ·016	0 17
15 min	30	0 ·04	0 38
After stimulation, calculated from cessation of stimulus—			
16 min	20	0 ·012	0 28
48 min	32	0 ·027	0 51
1 hr 40 min	30	0 012	0 15
2 hr 25 min	20	0 ·018	—
3 hr 30 min.	17	0 ·012	0 ·08
4 hr 47 min	15	0 004	(0 ·02)
6 hr 0 min	15	0 002	(0 ·00)
7 hr 45 min	8 5	0 ·004	0 07

Figures in brackets are inside the range of experimental error

In order to ascertain how much of the observed exudation was retained in the muscle, the muscle was weighed at the end of the experiment, *i e.*, 8 h. 15 m after the stimulus ceased The muscle on the opposite side was also weighed, its nerve having been cut 8 h 30 m previously.

		grm.
(A)	Weight of stimulated muscle	38·2
(B)	„ unstimulated muscle .. .	31·6
	Approximate quantity of fluid retained	6 2 or 19 p.c. of (B).

The muscles were also weighed in Ringer's solution, with the result that their specific gravities relatively to Ringer's solution were—

(A)	Sp gr. of stimulated muscle	1·061
(B)	„ unstimulated muscle	1 070

This difference in the specific gravity makes it clear that the difference in weight of the stimulated muscle was not to be accounted for by a difference in the original size of the stimulated muscle or a greater quantity of blood in it.

The observations on exudation, oxygen intake, and blood are typical of other experiments, except in so far as the effects do not always last so long, the minimal period of hyperæmia being two hours.

ii *The Submaxillary Gland.*

In most experiments pilocarpine has been used as a stimulant.

The following data are those of a typical experiment.—

Time	11 35	11 55	12 5	12 30	1 0	1 30	2.30	3 10	4 39	5 32
Blood, flow c c per min	2 3	10	4 8	6 7	6	6	4·8	3 7	3 4	3 3
O_2 in c c per min.	0 27	0 56	0 47	0 45	0 45	0 54	—	0 34	0 39	0·22
Total exudation in c c per min	0	2 6	1 12	0·63	0 31	0·413	0 54	0 26	0·23	0·12
Saliva in c c per min	0	1 37	0·68	0·63	0 30	0·35	0 32	0 21	0 19	0·09
Lymph, etc, in c c per min.	0	1 23	0 44	0	0·01	0·063	0·022	0·049	0 044	−0·027

The following points are important —

(1) The lymph, roughly speaking, varies with the quantity of saliva secreted [5]

(2) Relatively to the rate of flow of saliva the increased oxygen consumption and flow of blood rise for 2–3 hours.

REFERENCES

1 Verzár, 'Journ Physiol,' vol 44, p. 243.
2. Haldane and Lorrain Smith, 'Journ. Physiol,' vol 25, p 332
3 Verzár, *ibid.*
4. Barcroft, 'Journ. Physiol,' vol 25, p. 789
5 Asher, 'Zeitschr f Biol.,' vol. 37, p 261

Functional Œdema in Frog's Muscle.

By M. Back, K. M Cogan, and A. E Towers.

(Communicated by J Barcroft, F.R S Received February 18, 1915.)

The following experiments resulted from an observation that after stimulation of the gastrocnemius muscle on one side that muscle was heavier by about 20 mgrm than the muscle of the opposite side.

Series 1 —To ascertain whether the above phenomenon was of constant occurrence eleven experiments were performed in this series, together with four control experiments

Method.—The frogs were pithed, the brain and spinal cord both being destroyed. Electrodes were attached to the *tendo Achillis* and under the nerve as it entered the muscle. Care was taken to avoid hæmorrhage as much as possible, but we were not always successful in this

The preparation was stimulated by single induction shocks at the rate of 40 per minute for 15 minutes The muscles from the two legs were then dissected out and weighed, usually within 15 minutes of the end of the period of stimulation.

The right and left legs were used indiscriminately.

The following results were obtained —

Weight of muscle.	Excess of weight of stimulated muscle	Gain of weight per cent by stimulated muscle
mgrm	mgrm	
409	7	1·7
314	−6·5	−2·2
160	11	8·0
194	12	6·0
206	2·5	1·2
285	−3 5	−1 1
260	3 7	1·7
260	9 5	3·6
283	7·9	2·6
263	23	9·2
285	−3 5	−1 1

Of these, the stimulated muscle was the heavier in eight cases and the lighter in three, and in these three the disparity was trifling as compared with many of the cases in which the stimulated muscle was the heavier.

The following cases are given for comparison in which neither muscle was stimulated.—

Left muscle.	Right muscle	Difference	Difference per cent
mgrm	mgrm	mgrm	
237 5	241	3 5	1 5
150 5	159	8 5	5 7
156	152 5	−3 5*	2 2
247	241	−7	2 4

* Minus sign means that left is heavier

Inspection of the muscle made it clear that those in which we obtained a considerable gain in weight were as a rule muscles in which the circulation was good, whilst in some of the others the circulation was obviously deficient, we therefore determined to modify our technique by killing the frogs in a different way

Series 2 —The brain, in front of the medulla, was destroyed by means of a special instrument for this purpose, the medulla and the upper part of the spinal cord were left uninjured, but the lower portion of the cord was pithed in order to prevent the possibility of contractions being induced reflexly in the unstimulated gastrocnemius

The circulation was clearly much more satisfactory in this series, the respiration in most cases being well maintained and the frogs appearing to recover rapidly from the shock.

The following controls were obtained in which neither muscle was stimulated —

Left muscle.	Right muscle.	Difference.	Difference per cent.
mgrm.	mgrm	mgrm	
340 ·5	340 5	0	0
146	147 5	1 5	1 ·0
116 4	118	1 ·6	1 3

Whilst in the experiments in which one side was stimulated the results were as follows :—

Weight of muscle.	Excess of weight in stimulated side	Gain of weight per cent.
mgrm.	mgrm.	
469	25	5 ·1
345	8	2 ·3
219 ·5	13 ·2	7 ·6
310	19 8	6 ·1
427	3	0 ·7

Series 3.—The comparison of these results with the controls left no doubt in our minds that the stimulated muscles really did gain in weight. In order to test the matter in another way we determined the specific gravities of the stimulated and unstimulated muscles by weighing them in Ringer's solution. Our argument was that if the muscles merely differed accidentally in size, their specific gravities would be the same Even if the difference in size was due to a greater amount of blood in the stimulated muscle, the specific gravities would be much the same on account of the relatively high specific gravity of blood If, on the other hand, the difference in weight was due to the stimulated muscles taking up water from the blood as an osmotic phenomenon, the heavier muscles should have the lower specific gravity

Average weight of muscle	Excess of stimulated muscle	Specific gravity of stimulated muscle	Specific gravity of unstimulated muscle.
mgrm	mgrm		
192	37·0	1·061	1·073
257	25·0	1·064	1·075
285	7·6	1·075	1·078
287	3·2	1·065	1·066
323	28·4	1·070	1·069

With the exception of the fifth, the differences in specific gravity bear out our contention. To these we may add some data from muscles which were tested at longer intervals of time after stimulation

Average weight of muscle	Excess of stimulated muscle	Specific gravity of stimulated muscle	Specific gravity of unstimulated muscle
mgrm	mgrm.		
437·5	7·2	1·069	1·075
456·4	5·8	1·069	1·072
281·6	6·1	1·067	1·069
394·6	14·4	1·068	1·071
227·4	27·6	1·073	1·067

Series 4.—In all the above experiments the shocks passed through the muscle, in order to exclude the possibility of some direct electrical effect on the blood vessels or on the muscular tissue, we performed another series of experiments in which the frogs were killed as before, by destruction of the cerebrum and basal ganglia, but the spinal cord was not pithed. The two sciatic nerves were dissected out in the thigh and were cut, one was ligatured and stimulated. This technique appeared very satisfactory, the dissection of the nerves entailing less bleeding than the pithing of the lower part of the cord

Weight of muscle	Excess of weight of stimulated muscle	Gain of weight per cent by stimulated muscle
mgrm	mgrm	
370·0	12·1	3·5
189·5	18·6	9·7
254·0	30·5	12·0

The specific gravities were determined in two cases —

Average weight of muscle	Excess of stimulated muscle	Specific gravity of stimulated muscle.	Specific gravity of unstimulated muscle.
mgrm	mgrm		
370·0	12·1	1·054	1·059
189·5	18·6	1·076	1·072

Series 5.—We have made some experiments, as yet incomplete, to determine the length of time which elapses before the œdema passes away. At present we can say that it is evident after six hours from stimulation, but the fluid appears to have become absorbed in 16 hours; between these wide limits we have no data at present:—

Weight of muscle	Excess of weight of stimulated side	Gain of weight per cent	Interval after stimulation	
mgrm	mgrm		h	m
254	30·5	12		30
257	25	10		45
370	12·1	3·3		60
192	37	19	1	30
235	7·6	3·2	1	40
189·5	18·6	9·7	2	0
322·6	28·4	9·2	2	0
237	3·2	1·4	2	20
438	7·2	1·6	3	10
456·4	5·8	1·3	4	30
281·6	6·1	2·2	5	0
394·6	14·4	3·6	5	15
227·4	27·6	12	6	0
275	−17	−6·2	16	45
135·6	−1·8	−1·3	17	0

Discussion of Results.—It is clear that the phenomenon which we have observed can only take place when the muscle is well supplied with fluid from some external source. Fletcher* observed a phenomenon which is

* 'Journ. Physiol.,' vol. 30, p. 414 (1904). The reader is referred to this paper for an excellent account of the literature of the subject, including the early experiments of Ranke.

probably the same when he discovered that fatigued frog's muscle swells up when placed in water, to a much greater extent than resting muscle He observed also that sufficient exposure to an atmosphere of oxygen restores to the muscle in a marked degree the osmotic character of resting muscle It might, therefore, have been supposed that a vigorous circulation of blood through the muscle would have prevented the swelling which we have observed The fact, however, appears to be that muscle is capable of out-running its oxygen supply with great ease, and this is probably especially true of frog's muscle, in which the opportunities afforded for the acquisition of oxygen are much smaller than in the case of mammalian muscle

In comparing our result with that of Fletcher, it must be borne in mind that his phenomenon was probably a purely osmotic one, ours may involve, or may not, also some change in the permeability of the vessel walls

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The Effect of the Depth of Pulmonary Ventilation on the Oxygen in the Venous Blood of Man

By J F Twort and Leonard Hill, F R S

(From the Physiological Laboratory, London Hospital Medical College, and Research Fund.)

(Received January 21, 1915)

We have sought to gain evidence as to whether the arterial blood is saturated with oxygen during its passage through the lungs when the breathing is shallow and the subject lying at rest. Incidentally we have made some observations on —(1) The effect of work (2) The local application of heat or cold on the gases in the venous blood.

As means have not been devised for obtaining safely samples of normal arterial blood from man, we have been obliged to content ourselves with samples of venous blood collected from the veins of the arm.

The samples have been collected for us with strict aseptic precautions by Dr James McIntosh, and in some cases by Dr Paul Fildes Their daily practice in collecting blood samples from patients has made our colleagues skilful in the technique of this small operation We owe them our best thanks for their help.

Neither ligature nor compression was applied to the arm. The needle of the syringe was passed straight into the vein, the arm of the subject rested upon the couch and remained covered with the sleeve until the moment of

collection, 0·1 c c of 1-per-cent sodium citrate solution having been placed in the syringe to prevent clotting, the blood was drawn up exactly to the 1 c c mark The analyses were made by means of the small Barcroft apparatus—the oxygen being displaced by ferricyanide

The subject rested for some minutes on the couch, and at the time of collection of the blood breathed through a meter so that ventilation of the lungs was recorded A mouthpiece was employed fitted with wide tubes and mica inlet and outlet valves The nose was closed with a clip One sample was collected while the subject breathed quietly, and another from the same, or the other arm, while he breathed forcibly. In the experiments in which oxygen was breathed the subject inhaled from a large bag filled with oxygen and exhaled through the meter

Double samples taken as controls under the same conditions gave us results which agreed within fairly close limits For example —

11 4 per cent, right arm, 12 0 per cent, left arm

Table I —Cubic Centimetres of Oxygen per 100 c c of Blood

Subject	Resting				Working	
	Breathing air quietly	Breathing oxygen quietly	Breathing air forcibly	Breathing oxygen forcibly	Breathing air quietly	Breathing oxygen quietly
1	12 0	8 6	—	—	5 5	6 3
2	5·9	9 5	-	-	—	3 2
3	7 6	9 7	—	—	6 8	9 2
4	12 7	13 8	—	—	7 0	6 8
5	2 6	5 0	—	—	2 8	4 7
6	14 0	14 6	—	—	8 2	7 3
7	10 4	8 5	—	—	3 3	1 5
8	8 8	—	—	—	6 5	—
9	11 4	—	—	—	—	—
	12 0	—	—	-	—	—
10	4 5	11 1	—	—	—	—
11	6 3	—	13 0	—	—	—
12	7 4	—	14 6	--		—
13	7 5	—	14 6	—	-	—
14	—	13·7	—	14 6	—	—
15	—	13 2	—	13 8	—	—
16	—	12 4	—	12 2	—	—
17	—	8 3	—	9 2	—	-
18	8 3	7 9	—	—	-	—
19	—	15 7	11·6	—	—	—
20	—	10 9	15 2	—	—	—
21	13 4	15·6	—	-	—	—
22	9 1	13 4	—	—	—	—
23	4·6	7 0	—	—	—	—
24	14 1	15 4	—	—	—	—
25	16·0	17·9	—	—	—	—
26	8·6	9 4	—	—	—	—
27	10 8	12 5	—	—	—	—

Considering the slight physiological differences which may arise, *e.g.*, from posture, exposure to cold during the collection of samples, etc., we cannot expect to get closer results. To carry out work the subject grasped a spring ergograph and squeezed it 20 times a minute, the sample was collected immediately at the end of two minutes period of work.

The average of 22 analyses taken when resting and breathing air quietly is 9·5 per cent, breathing oxygen quietly 11·8 per cent.

The average of analyses when working and breathing air quietly is 5·7 per cent, while that when working and breathing oxygen quietly is 5·6 per cent. The corresponding resting analyses in the same subjects gave 9·4 per cent when breathing air quietly, 10·0 per cent when breathing oxygen quietly.

Table II.—Effect of Warming One Arm.

Subject	Arm not warmed	Arm warmed with bath	Remarks
	per cent.	per cent.	
1	9·4	14·8	Air quietly breathed
2	4·8	11·7	" "
3	10·0	11·6	" "
4	10·8	12·2	Oxygen quietly breathed
5	12·6	14·2	Air forcibly breathed
6	15·2	16·4	Oxygen breathed quietly

The average of the six experiments is 10·3 per cent. for the unwarmed and 13·5 per cent. for the warmed arm.

Table III.—Effect of Forcibly Breathing Air or Oxygen.

Subject	Forcibly breathing air	Forcibly breathing oxygen
	per cent.	per cent.
1	14·2	14·8
2	11·3	11·8
3	13·5	13·1
4	12·9	12·4
5	9·2	9·8
6	17·1	16·8
7	14·1	14·1
8	14·2	16·0
9	11·6	—
10	15·2	—
11	13·0	—
12	14·0	—
13	14·6	—

The average of 13 analyses of samples taken when forcibly breathing air is 13·5 per cent., and of eight breathing oxygen forcibly 13·6 per cent.

To sum up, then, the average results of the analyses are:—

No of analyses	Cubic Centimetres Oxygen
22	9·5 resting, breathing air quietly
22	11·8 resting, breathing oxygen quietly
7	5·7 working, breathing air quietly
7	5·6 working, breathing oxygen quietly
3	7·9 resting, breathing air quietly, arm not warmed
3	12·7 resting, breathing air quietly, arm warmed (to produce vaso dilatation)
3	12·7 resting, breathing oxygen quietly (2), air forcibly (1), arm not warmed
3	14·2 resting, breathing oxygen quietly (2), air forcibly (1); arm warmed
13	13·5 resting, breathing air forcibly
8	13·6 resting, breathing oxygen forcibly

Our subjects were medical students and laboratory servants Some of them, when lying on the couch and breathing quietly, gave us low, and some high readings The difference is an individual one, and cannot be ascribed to errors in technique, for he who gives a low reading when resting gives a low reading when working Moreover the deep breathing readings are uniformly high Some of our subjects were emotionally affected by the operative procedure and breathed about 10 litres a minute, while others breathed only 5–6 litres, while resting on the couch. We cannot, however, ascribe the higher reading in all cases to the ampler breathing All we can affirm is that quiet breathing gives us a certain proportion of low readings in the given number of subjects, while deep breathing gives us uniformly high readings

Looking at the difference between the average figures for resting and breathing air quietly and breathing oxygen quietly it might be assumed that this was due to the oxygen simply dissolved in the blood according to the law of partial pressures If pure oxygen were breathed we might expect a little over 2 per cent O_2 to be simply dissolved, and the tissues, using this oxygen first, would dissociate the hæmoglobin less by the same amount

The subjects were breathing not 100 per cent. but about 80 per cent of oxygen, so the amount simply dissolved would not be quite as much as 2 per cent When, however, we compare the figures obtained during forcible breathing of air or oxygen, we see no evidence of any excess of oxygen due to simple solution under the increased partial pressure of this gas

Similarly in a very careful series of analyses of cat's blood recorded by Buckmaster and Gardner and obtained by means of the Topler pump we see no evidence of any increase in oxygen of the arterial blood due to the breathing of oxygen in place of air. The average of 13 analyses, the cats breathing air, was 14·2 per cent, breathing oxygen 14·9 per cent.

The theoretical oxygen capacity determined from the hæmoglobin value was about 17 per cent These authors say —

"From the experiments it is a fair conclusion that during its passage through the pulmonary capillaries the blood is rarely fully saturated with oxygen even when oxygen is inhaled For an explanation, it is probable that parts of the lung, for example the apices, are imperfectly ventilated, and also, since the circulation time in the lung is only about five or six seconds, that complete equilibrium is not attained between the blood and alveolar air "*

We know that anything over 75 per cent of an atmosphere of oxygen when continuously breathed produces pneumonia, and that exposure to two or three atmospheres of oxygen causes convulsions A high partial pressure and concentration of oxygen on the blood acts as a poison It may be that there is at work some mechanism which prevents, within certain limits of oxygen partial pressure, the over-concentration of free oxygen in the blood, and therefore we find no more oxygen in the venous blood on forcibly breathing air than on forcibly breathing oxygen

Our figures show that forcible breathing of air, or oxygen, equally and notably increases the oxygen in the venous blood above the average result obtained when breathing air quietly We cannot ascribe this result to vaso-dilatation and accelerated flow through the arm produced by the forced breathing, for G N Stewart has shown that forcible breathing diminishes the velocity of flow in the hand by about 40 per cent.† Forcible breathing mechanically interferes with the circulation and the hand tends to become pale and cold when such is continued

We conclude that the arterial blood is not always saturated with oxygen during the passage through the lungs when the breathing is quiet Some parts of the lung may remain unexpanded, and the blood passing through these parts is not oxygenated Forcible breathing ensures the expansion of all parts and the better saturation of the arterial blood In one case Caske and Barcroft‡ obtained a sample of arterial blood and found it 94 per cent saturated with oxygen The sample was obtained from a young woman acting as donor in a direct transfusion of blood Her artery was opened under local anæsthesia. The emotional conditions probably ensured in her a good pulmonary ventilation.

If it be true that the person engaged in sedentary occupation does not expand the lungs sufficiently to arterialise the blood in all their parts, this

* 'Roy Soc Proc,' B, vol 85, p 56 (1912)

† 'Amer Journ Physiol,' vol 28, p 190 (1911).

‡ 'Proc. Physiol. Soc.,' 'Journ Physiol.,' vol. 47, p. xxxv (1914)

may be a contributory cause of a lessened immunity to the organism of disease such as phthisis.

Our results too confirm the need for caisson workers not to rest during decompression but to take exercise and to breathe deeply so as to secure the escape of nitrogen, which has been dissolved in their body fluid during their work in compressed air

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On the Occurrence of an Intracranial Ganglion upon the Oculomotor Nerve in Scyllium canicula, *with a Suggestion as to its Bearing upon the Question of the Segmental Value of Certain of the Cranial Nerves.*

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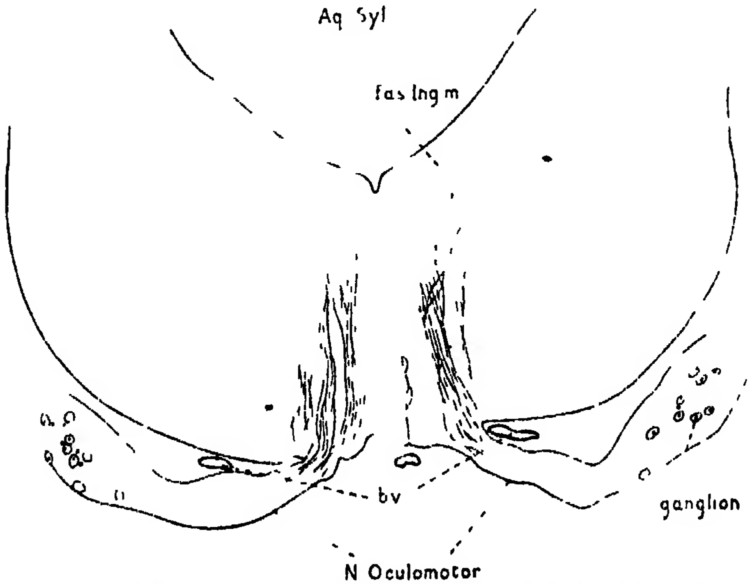
During the study of a number of elasmobranch brains made in connection with my work on Reissner's fibre, I noticed, in a specimen of *Scyllium canicula*, a collection of ganglion cells upon a length of nerve lying freely beneath the mid-brain This particular brain had been sectioned in the longitudinal vertical plane and the ganglionic mass occurred at a place which corresponded with the level of the third cranial nerve Further examination showed that these cells were undoubtedly related to the oculomotor nerve They are situated upon it in a scattered group which, beginning at a point about 1 4 mm. from the superficial origin of the nerve, stretches to its severed end (roughly 1 6 mm. from its origin). The cells, though only about 15 in number, are moderately large (averaging $20\mu \times 18\mu$) and are apparently unipolar or bipolar Their distribution suggested that other cells of the group must have existed distally to the point of severance of the nerve.

Upon the opposite side of the brain the corresponding nerve had been cut away quite close to its superficial origin, when the brain was removed from the cranium.

A second specimen of *S canicula* in which some 2 mm. of the third nerve had been left attached to the brain, on either side, showed the ganglion well on both nerves.

This specimen had been sectioned in the transverse plane and is the one from which the text-figure has been drawn. The cells are seen on either side,



Lower part of a Transverse Section through the Brain of *Scyllium canicula*, taken at the level of the origin of the oculomotor nerves, and showing some of the ganglion cells slightly diagrammatically.
(Outline with camera lucida. × 14 approx.)

at about the same distance from the superficial origin of the nerve, in a group extending between points roughly 1.5 mm. and 2 mm. from that origin. On the one side some 50 odd ganglion cells were counted, while upon the opposite side there were about 70. The exact numbers cannot be certainly stated, for it is probable that in some cases a cell may have been reckoned twice, appearing, as they do, each in several adjacent sections. They were accompanied by smaller cells, the nuclei of which stained much more deeply, the whole forming a quite obvious ganglion.

My remaining series of *S. canicula* brains, in every case, showed the third nerves cut at a point nearer to the brain than that at which the ganglia might be expected.

Another series, therefore, was cut transversely specially for this investigation, care being taken to remove the brain with as much as possible of the third nerves attached. The ganglionic masses were found well marked and were composed of cells of the same character and in about the same number as in the case described and figured above.

The discovery of even a small ganglionic mass related to the third cranial

nerve was so unexpected that I was led to examine other elasmobranch brains of which I had sections, with a view to determining whether the condition in *S. canicula* was peculiar to that species or whether the ganglion was of normal occurrence but had escaped observation

The examination was without result, however, for in a number of specimens of *Acanthias, Raia*, and *Rhina* I found no trace of ganglion cells upon the third nerve root, but it cannot be asserted that in these species the ganglia are absent, for in all of these brains the third nerve had been severed, it was found, comparatively close to its origin A search for these cells upon the third nerve of Amphibia was somewhat more fruitful In *Rana esculenta* a cluster of half a dozen large ganglion cells was found upon the nerve on one side of my single specimen In *R temporaria* and *Molge sp* an odd cell or two appeared in the sections of the nerve In each case the cells were found at or very near to the severed end, and it is highly probable that still other cells had existed in the distal part of the nerve

The existence of these ganglia in *S canicula* has not, I believe, been recorded hitherto This is, perhaps, not surprising, for the head of the adult animal is far too large to be readily cut in its entirety, and, as my own experience illustrates, where a study of the central nervous system is the prime object, brains are likely to be removed from the head by the cutting of the third nerves fairly closely to their superficial origin And, in sections of the entire heads of embryos of this species, of several different sizes, which I examined, I was unable to distinguish the future ganglion cells among the numerous cellular elements present in the developing third nerve, although some of my specimens were sufficiently advanced to show the elements of the trigeminal ganglion already fairly well differentiated.

Although I have seen these ganglia in but three specimens of *S. canicula* I believe that they will prove to be actually of invariable occurrence in this species

That "degenerated" ganglion cells occur upon the oculomotorius in man has long been known, having been recorded by Thomsen in 1887.* They were said to occur in the adult human subject as patches of altered tissue which, Thomsen stated, were the remains of the encapsulated ganglion cells observed by him in the root of the oculomotor in the new-born child Gaskell (89), two years later, confirmed the discovery of these degenerated cells in the third nerve of man and recorded the finding of similar cell masses in the root of the fourth and sixth nerves also He seems to have assumed that these cells were always merely vestigial structures, but he nevertheless attached considerable importance to the discovery. On the strength of the transient

* *Vide* Tozer, '12.

existence of these cells he put forward the hypothesis that "both third and fourth nerves are in themselves complete segmental nerves of the type which Balfour supposes to have been the original type 'when mixed motor and sensory posterior roots were the only roots present', that then owing to some change which occurred during the past history of the vertebrate the sensory parts of these two nerves degenerated and their place was taken by the sensory elements of the fifth nerve"

Two other early observers, Reissner and Rosenthal,* are also said to have recorded the existence of cells in the root of the third cranial nerve According to Sherrington, the former suggested that these cells were connected with the sympathetic system

Of the occurrence of (presumably) actively functional ganglion cells upon the oculomotor root, in numbers comparable to those which I have found in *S canicula*, the only record, so far as I can discover, is contained in the paper by Miss Tozer ('12) This author found ganglion cells in (or near) the roots of the third, fourth, and sixth cranial nerves of young *Macacus rhesus* and of a teleost (*Gadus virens*) and in the third and sixth roots of the common pigeon

It would appear, then, that oculomotor ganglia† are present, either as functional structures or as vestiges, in widely separated vertebrate classes Since ganglia, other than sympathetic, are known to occur normally only upon "sensory" nerves, or if upon mixed (motor and sensory) nerves, then upon the dorsal (sensory) root only, a question at once presents itself—What is the significance of the occurrence of ganglia upon the oculomotor root?

In seeking an answer to this question it will be necessary to discuss the generally accepted belief in the homology of the oculomotor nerve with a ventral spinal root in the light of our present knowledge of (*a*) the composition of this nerve and (*b*) its relation, in development, to the ciliary ganglion

The Comparison of the Oculomotor with a Ventral Spinal Nerve Root

That the oculomotor has, of recent years, been accepted by the great majority of authors as a purely motor nerve, composed only of somatic motor components and equivalent simply to the ventral root of a typical segmental nerve is common knowledge. Neal ('14), the most recent contributor to this question, has insisted upon the correctness of this interpretation. He

* *Vide* Sherrington, '94*a*, p. 254

† These ganglia (which occur on the roots of the oculomotor just within the brain case) must not be confused with the ciliary ganglia which have sometimes been referred to as oculomotor ganglia The ciliary ganglia, of course, lie in the orbit and, although connected with the oculomotor (and ophthalmicus profundus) nerves, are generally recognised as being ganglia properly referred to the autonomic (sympathetic) system.

concludes* "the evidence of its histogenesis and its central and peripheral relations so strongly support the supposition that it is a somatic motor nerve, as the majority of morphologists have believed, that the acceptance of the latter seems unavoidable"

While Neal is undoubtedly justified in claiming that this view is that held at the present time by the majority of observers, yet, as he points out, this has not always been the case In the past, many morphologists have maintained that the oculomotor is serially homologous with the segmental nerves Comparatively recently this earlier view has been supported by Gast ('09) upon embryological grounds

In arriving at his conclusion, however, Neal appears to be altogether unaware of the light which has been shed upon this question by the results of the experimental work of Sherrington and Tozer.

The Composition of the OculomotorNerve—Sherrington ('94), as the result of certain experiments upon the eye-muscle nerves, came to the conclusion that the oculomotor might prove to be "sensori-motor" (afferent-efferent) He repeated and laid stress upon this suggestion in a later paper ('97)

Herrick ('99, p 230) noted that medullated nerve fibres of two kinds were to be recognised in the oculomotor nerve of a bony fish (*Menidia*) He remarked that muscle-spindles were said not to occur in the eye-muscles but suggested that the more slender nerve fibres, whose origin he failed to determine, might, nevertheless, form part of a sensory mechanism analogous with muscle-spindles

Recently the existence of muscle-spindles in the extrinsic eye-muscles has been demonstrated by Sherrington and Tozer ('10) Not only so, but the related spindle nerves were stated to pass into the central nervous system by way of the oculomotor This, upon the generally accepted interpretation of the oculomotor nerve as merely a ventral root, is altogether anomalous, for the nerve fibres from muscle-spindles in all other muscles are derived from ganglion cells of the dorsal spinal ganglia and thus are connected with the central nervous system only by way of the dorsal (posterior) nerve roots

The experimental work carried out by Miss Tozer ('12) upon *Macacus* showed that lesion of the third nerve peripheral to the ganglion cells (*i.e.*, at a point between the ganglion cells and the muscle-spindles) resulted in the alteration of the ganglion cells and the complete degeneration of the muscle-spindles; whereas, when the lesion was effected centrally to the ganglion, some† at least of the muscle-spindles persisted

* *Op cit.*, p. 105

† It should be noted that while only a few muscle-spindles are said to have persisted, in *Macacus* only a few ganglion cells are normally present.

This is not only definite confirmation of Sherrington's suggestion that the third nerve is sensori-motor, but it also points to a connection between these ganglionic cells and afferent fibres from sensory end-organs (the muscle-spindles) It thus affords evidence that these cells are of a type normally occurring elsewhere only upon the dorsal roots of segmental nerves

In summing up, Miss Tozer remarks* "The great variation in the number of these cells renders an explanation of their nature and function at present impossible The cells in *Gadus* are possibly sufficiently numerous to represent the source of the afferent fibres . . but in *Macacus* the number of these cells seems to be insufficient to do this" If the latter part of the statement is correct, some of the afferent fibres presumably have an intracerebral origin

Since the oculomotor nuclei are in close relation to several sensory centres through the mediation of the fasciculus longitudinalis, there is nothing improbable in the suggestion that some of the afferent fibres may pass directly, by the fasciculus longitudinalis, to one of these centres

The Relation, in Development, of the Ciliary Ganglion to the Oculomotor — Carpenter ('06, p 192) has demonstrated that while, in the embryo chick, the ciliary ganglion arises, in part, from cells which have migrated from the ophthalmicus profundus ganglion, it is also, to some extent, derived from neuroblasts which pass peripherally along the fibres of the oculomotor nerve to the ciliary ganglion

In view of this statement I was, at first, inclined to believe that the oculomotor ganglia which I had observed were merely cell masses destined primarily for the sympathetic system but which had been arrested at this place in their transit to the ciliary ganglion Such an explanation, however, merely introduces another difficulty, viz, the apparently anomalous origin of the ciliary ganglion

In a typical segmental nerve, sympathetic ganglia are generally believed to arise by the migration of neuroblasts from the spinal ganglion upon the dorsal root † The alternative view that sympathetic ganglia arise, either wholly or in part, by the migration of medullary cells along a ventral nerve root has met with little acceptance The former view is that favoured by Neal In an earlier work, that author maintained ('03) that, although the cells in the anlagen of the spinal ventral roots had a medullary origin, yet these had nothing to do with the formation of neuraxons They are differentiated solely into neurilemma elements In his recent work he notes ('14, p. 54) that this fact has never been called in question but that, on the contrary, it has been confirmed by several subsequent workers.

Concerning Carpenter's statement that neuroblasts pass along the oculo-

* *Op. cit*, p. xvi

† *Cf.* Johnston, '07, p. 206.

motor to the ciliary ganglion, Neal says ('14, p 73) "If this conclusion be confirmed—and this has been done by Belogolowy—it appears that at least some of the medullary cells in the oculomotorius anlage are neuroblastic."

Belogolowy ('10) apparently only infers, however, that the cells of medullary origin in the anlage of the oculomotorius are those which later enter the ciliary ganglion.

Gast ('09) believes that, in the embryo, there exists a "root-ganglion" upon the oculomotor nerve, the cells of which are of medullary origin Concerning this, Neal points out the difficulty of recognising with certainty a nerve cell among the mass of cellular elements in the anlage of the nerve, a difficulty to which I have already alluded *

Moreover, many of these cells of medullary origin are known to become altered later into neurilemma elements, as do the similar cells in spinal nerves Carpenter has observed all stages in the differentiation of such medullary cells into neurilemma, in the chick Neal records that he, too, has seen such a transformation, in embryo *Squalus* His statement ('14, p. 74) is, ". in Squalus, it is possible to demonstrate that a large number, if not all, of the cells present in the anlage of the oculomotor become differentiated as neurilemma cells."

In this connection Neal reviews the evidence bearing upon the question To what extent, if at all, do emigrating medullary elements contribute to the formation of the sympathetic ganglia related to spinal nerves? The work of Kuntz ('11) (which Neal remarks is "the latest presentation of the case in favour of the medullary origin of some of the elements of the sympathetic") comes in for somewhat severe criticism Neal concludes that convincing evidence "that cells of somatic motor nerve anlagen in Squalus migrate into the anlagen of the sympathetic .is wanting The assertions of Kuntz in this connection appear quite unconvincing"

As the result of his own observations Neal expresses the opinion ('14, p. 57) that "the evidence seems rather to favour the view that the sympathetic anlagen receive their cellular elements largely, if not exclusively, from the sensory ganglia, as inferred by investigators upon all classes of vertebrates from Schenck and Birdsall to Held and Marcus"

Thus, in contributing to the formation of the sympathetic (ciliary) anlage the oculomotor would, as a ventral root, appear to be altogether exceptional In the anlage of the oculomotorius, however, there are present cells which are derived from the neural crest

The statement that free cell migration takes place from the mesocephalic ganglionic mass towards the oculomotor anlage has been made by numerous

* *Vide* p 555, *supra*

observers Recently Carpenter ('06), Gast ('09), and Belogolowy ('10) have confirmed this, but the two latter authors suppose that these migrant neural crest cells simply become neurilemma and supporting cells in the oculomotor root

Johnston ('05, p. 244) believed that the cells of the ciliary ganglion arose from a part of the neural crest distinct from that which gave rise to the profundus ganglion Neal denies that this is true for *Squalus*, but makes a very interesting statement concerning the origin of the ciliary ganglion in that form " . the first clusters of cells associated with the anlagen of these nerves (the oculomotor and the trochlear) are derived from the neural crest. These cell clusters, in their relations and—in the case of the ciliary—in their adult structure, appear to be sympathetic Their derivation from the neural crest favours the inference that the sympathetic anlagen of the trunk have a similar origin" ('14, pp. 58–59).

From all of which, indefinite and conflicting as it appears, three facts emerge:—

(i) that, in the anlage of the oculomotor, cells are found derived by migration (*a*) from the medulla, and (*b*) from the neural crest,

(ii) that certain of these cells in the oculomotor anlage migrate into the anlage of the ciliary ganglion, precisely as do cells from a typical dorsal ganglion into a typical sympathetic ganglion,

(iii) that the weight of evidence appears to be against the belief that cells of medullary origin contribute to the formation of sympathetic ganglia.

The inference is that the cells which pass along the oculomotor to the ciliary ganglion must have been derived, in the first instance, from the neural crest.

The Comparison of the Oculomotor with a Complete Segmental Nerve

In view of what has been said it will be obvious that, in certain particulars, the peripheral relations of the oculomotor are not exactly those of a ventral root. Nor does it appear that the histogenesis of this root (in its relation to the neural crest and the ciliary ganglion) corresponds, in every particular, with that of a spinal ventral root In attempting to arrive at a correct interpretation of the homology of the oculomotor we must take into account the following facts —

(i) that this nerve is undoubtedly "afferent-efferent", some or all of its afferent fibres being related to sensory end-organs (muscle-spindles);

(ii) that, upon its root, in some forms, occurs a ganglion or scattered ganglion cells;

(iii) that, in such forms it is probable that the afferent fibres are wholly, or in part, derived from these ganglion cells ;

(iv) that, in development, ganglion cells from the neural crest enter into association with the anlage of the oculomotor, apparently establishing a transient dorsal root ;

(v) that, in development, certain ganglion cells migrate peripherally along the fibres of this nerve to the (sympathetic) ciliary ganglion, such cells having probably arisen primarily from the neural crest

Now in a typical segmental nerve (in the general acceptation of the term) we find a dorsal root bearing a ganglion and a ventral root without ganglion cells These roots unite at, or just peripheral to, the ganglion From the ganglion, or from the common nerve distal to the ganglion, there arises a branch which enters a ganglion of the autonomic nervous system The cells of this latter ganglion are derived predominantly, if not exclusively, in embryonic life, from cells which migrate from the spinal ganglion situated upon the dorsal root of the related segmental nerve The nerve fibres of the dorsal root are, in general, derived from the cells of its dorsal ganglion and are described as afferent (somatic and visceral sensory) fibres, transmitting centripetal impulses The nerve fibres of the ventral root are of intraspinal origin (arising from the ventral and lateral cornua of the spinal cord), and are efferent (motor), transmitting only centrifugal impulses.

In a comparison of the condition observed in the oculomotor with that defined as typical for a segmental nerve we cannot but be struck with the fact that the oculomotor appears to combine most of the features of dorsal and ventral roots of the typical segmental nerve. That it may lack certain nerve components, normally present in a typical segmental nerve, is not denied, nor is a distinct dorsal root recognisable. On the other hand it presents features altogether unknown in any typical segmental ventral nerve root

The Absence of Certain Components from the Oculomotor.—The suggestion that there has occurred in this nerve a cenogenetic atrophy of certain components is neither new nor improbable. Indeed, Johnston has pointed out ('07, pp. 151–153) that, in the nerves of that region of the head occupied by the lateral eyes, the absence of the general cutaneous component of the sensory system might reasonably be expected. Similar reasoning might connect the absence of visceral sensory components with a possible disappearance of visceral tissue in the region of the more anterior somites. With the disappearance of these nerve elements from the nerves of the segments occupied (or encroached upon) by the lateral eyes and the serially

following "accessory optic vesicles," the dorsal roots would undergo considerable diminution in size and importance The dorsal ganglia, from which most of the remaining afferent fibres would have arisen, would consist of comparatively few cells (the large contingents of cells, normally present in ganglia upon nerves supplying regions of less specialisation, being altogether wanting).

Gast has stated that a transient dorsal root, related to the oculomotor, appears in development, and considers that this and other eye-muscle nerves represent segmental nerves from which the sensory elements have altogether disappeared

Neal, dissenting entirely from Gast's speculation, remarks ('14, p 105) "The supposed demonstration of the participation of sensory elements in the genesis of the oculomotor is one that would satisfy only on the basis of a strong presumption in its favour" I would suggest that the actual occurrence, in *Macacus rhesus*, *Columba livia*, *Gadus virens*, and *Scyllium canicula*, of numerous ganglion cells in the oculomotor roots, apparently related to afferent nerve fibres connected with sensory end-organs, supplies the "strong presumption" which Neal requires Neal continues, "The position of the nidulus of the oculomotor and its peripheral distribution create a strong presumption against the assumption Spindle-shaped cells lying in the mesenchyma between the profundus ganglion and the oculomotor nerve are not necessarily neuroblasts Spindle-shaped cells may be found almost anywhere in the mesenchyma Even if it be admitted that the evidence that these cells are in the process of migration towards the oculomotor anlage is convincing, Gast does not know their fate They may form neurilemma or they may enter the sympathetic or what-not" Is it not reasonable to suppose that the cells of the oculomotor ganglion may represent some of the sensory elements which take part, according to Gast, in the formation of the oculomotor anlage ?

If others of these cells migrating to the oculomotor should subsequently "enter the sympathetic" as Neal suggests, it would strengthen rather than weaken the case for regarding the oculomotor as a segmental nerve. Neal himself states ('14, p. 57) that neural crest cells do come into connection with the oculomotor anlage and do apparently, amongst other destinations, arrive ultimately at the sympathetic (ciliary) anlage Indeed, Neal makes use of this fact to support his contention that spinal sympathetic ganglia have an origin from dorsal spinal ganglia, by analogy with this development of the ciliary ganglion

The Displacement of Dorsal Roots.—The dorsal roots of cranial nerves practically always shift ventrally from their primary position, during the

course of development This ventral dislocation may easily have been carried to a greater length in the case of a greatly reduced oculomotor dorsal root and have resulted in a confluence of both dorsal and ventral roots into a single structure

The Structure of the Oculomotor unlike that of Ventral Spinal Roots—The existence in the oculomotor of sensory fibres (related to muscle-spindles) derived in part, if not entirely, from ganglion cells upon the root of the nerve is absolutely incompatible with the interpretation of the oculomotor as homologous with a ventral spinal nerve root That some of the afferent fibres may have (as Tozer implies) an intracerebral origin does not affect the case Sherrington ('97*a*, p 210) states that, in mammals, none of the fibres of dorsal (posterior) spinal roots have an intraspinal origin For many vertebrates, other than mammals, however, it appears to be established that afferent fibres with an intraspinal origin do exist Thus the possible occurrence of afferent fibres of intracerebral origin in the oculomotor does not lessen the resemblance of this nerve to a true segmental nerve What is of the utmost importance is the indisputable fact that afferent fibres enter the brain by the root of the third nerve Of the spinal or typical segmental nerves Tooth concludes ('92, p 783) "The posterior roots are the only points of entrance of sensory, or, more broadly, centripetal impressions"

The explanation which appears to be most in agreement with the facts is that the oculomotor is not correctly viewed as the equivalent of a ventral root only Rather we must accept it as the homologue of a complete segmental nerve, containing elements of both dorsal and ventral roots, although some of these components have apparently become obsolete, and the distinction of the originally separate dorsal and ventral roots has disappeared

Against this hypothesis, which, it should be noted, is not quite identical with that put forward by Gaskell and earlier observers, three objections may be raised. Equally with that earlier view it is opposed to the generally accepted interpretation of the ramus ophthalmicus profundus as the dorsal nerve root of the oculomotor neuromere.

In the first place, then, it may be urged that, in ontogeny, the oculomotor and profundus nerves are very intimately related, and that the connection of the latter nerve and the neuromere of the oculomotor (v, according to Johnston) is only lost relatively late in development, when the profundus acquires a new connection with the brain (in neuromere vii) through the mediation of the trigeminal root Indeed, the ramus profundus retains its relation to the third nerve, throughout life, by the ciliary nerve (radix ciliaris longus) and ganglion.

It seems to me that the only inference which can safely be drawn from the observed shifting of the ophthalmicus profundus is that its change of relations from the third nerve to the fifth may recapitulate a change which took place comparatively late in the history of the development of the vertebrate head It does not, however, justify the assertion that the relation of the ophthalmicus profundus to the third nerve was necessarily primary The mesocephalic neural crest has a considerable antero-posterior extension and other connections between the neural crest and the oculomotor have been observed, anterior to that existing between this nerve and the ophthalmicus profundus

That the trigeminus, the ophthalmicus profundus, the eye-muscle nerves, and the nervus thalamicus, should be found related and more or less fused, perhaps shifted, or even become obsolete, is little to be wondered at, occurring as they do in a region where shifting and obliteration of myotomes has, admittedly, been such a marked feature Nor is it surprising that there remains little or no evidence, in ontogeny, of the primary arrangement in serial independence of the nerves of this region, for the changes which took place in connection with the development of the eye must have been some of the very earliest to disturb the serial arrangement of the nerves

In the nerves of the branchial region we have, apparently, a nearly parallel case There, although we find the several branchial nerves connected from the earliest developmental stages, yet it is generally accepted that these nerves were primarily independent and serially distinct It is assumed that their displacement and fusion was a feature acquired so early in the development of the vertebrate head that the prior condition no longer occurs in an abbreviated ontogeny

Bearing upon this hypothesis that the ophthalmicus profundus does not represent the dorsal root of a segmental nerve to which the oculomotor would stand merely in the relation of a ventral root, an interesting point may be noted In certain elasmobranchs, of which *Scyllium* is one, the ophthalmicus profundus is said to be little developed* and in the adult to be absent † It is precisely in *Scyllium*, where the oculomotor is found retaining its ganglion, that the encroachment of the ophthalmicus profundus is thus least in evidence

As a further objection, the connection of the ciliary ganglion with both the oculomotor and the ophthalmicus profundus nerves might be adduced, on the assumption that this is homologous with the relation, in the spinal region, of

* Sedgwick, '05, p. 135

† Parker and Haswell, '10, vol. 2, p 161, footnote

a sympathetic ganglion to both the ventral and dorsal roots of its related spinal nerve

Neal, discussing the question of the relation of the oculomotorius to the ramus profundus, decides ('14, p. 102), "there appears to be no insuperable objection to the view that the ophthalmicus profundus is serially homologous with spinal somatic sensory nerves" While this is readily conceded, it falls short of establishing that the ramus profundus necessarily represents the sensory root in the oculomotor neuromere Neal continues "The comparison of the profundus nerve with spinal somatic sensory nerves is still further strengthened by the evidence of the relations with the ciliary ganglion, which have been found above to be those of a somatic motor nerve to a sympathetic ganglion The facts which prove the sympathetic character of the ciliary anlage have already been stated above and need no restatement The ciliary ganglion of Squalus is to be regarded as partly, if not exclusively, a sympathetic ganglion So that in its relations with a sympathetic ganglion the oculomotor forms no exception in the series of morphologically similar somatic motor nerves"

From what we know of the development of the ciliary ganglion, however, this relation may, quite as reasonably, be interpreted upon the hypothesis of the segmental distinctness of the two nerves In accordance with which, I suggest that the ciliary ganglion is to be regarded as the product of the fusion of sympathetic ganglia related to at least two segmentally distinct cranial nerves Such a condition is paralleled in the spinal region in the cervical ganglia, for example Even if we admit the correctness of Krause's view that the ciliary ganglion has a double nature (containing, in addition to its sympathetic elements, the bipolar cells of a cerebro-spinal ganglion), the comparison of the oculomotor nerve with a typical segmental nerve would not be affected We should merely recognise that certain cells, migrating to the ciliary ganglion along the fibres of the oculomotor and ophthalmicus nerves, which were hitherto supposed to be simply sympathetic cells, were, in fact, sensory cells

It may well be that the ciliary ganglion is not strictly homologous in all species. The oculomotor ganglion has been observed in but comparatively few species, and, while its presence will probably be revealed by further investigation in many other forms, yet it is scarcely credible that it can have been completely overlooked in many types which have been carefully studied In such forms, then, the cells of the oculomotor ganglion may have migrated into the proximity of, or even into actual fusion with, the ciliary ganglion, which would thus have the double character claimed for it by Krause On the other hand, in those species in which a distinct oculomotor

ganglion persists, the ciliary ganglion may prove to be composed strictly of sympathetic elements.

Herein, perhaps, lies an explanation of the contradictory observations which have been recorded, and the diverse opinions expressed in the controversies concerning both the nature of the ciliary ganglion and the segmental value of the oculomotor nerve

The fact that ganglion cells are known to occur upon ventral spinal roots, constantly in the cat,* and occasionally in man and monkey, must not be overlooked These cells are not, however, associated with afferent fibres† nor related to sensorial end-organs What their nature and function may be has not yet been explained, but they are clearly not comparable with the cells found in the roots of the eye-muscle nerves Neal ('14, p 58) remarks, in connection with the migration of medullary cells into the oculomotor and trochlear nerves, that a similar migration is observed in the case of the abducens which has no related sympathetic anlage, and that in this case these cells can have no destination other than the neurilemma In the specimens which I have examined, ganglion cells were apparently absent from the roots of the fourth and sixth nerves. From the results obtained by Gaskell, Sherrington and Tozer, however, it would seem that both of these nerves also are sensori-motor, and have ganglion cells upon their roots in some species.

In the case of the trochlear nerve, Neal points out that, in development, it is closely associated with fragments of the neural crest and is related to a transient sympathetic ganglion The abducens undergoes considerable dislocation, and has lost all trace of any relation to a sympathetic ganglion anlage, if such ever existed. Nor has any connection between this nerve and the neural crest been recorded. Nevertheless, it would seem that the arguments adduced in favour of the segmental value of the oculomotor would apply, in the main, to all the eye-muscle nerves, making allowance for the progressively greater reduction and displacement which has occurred in the two more posterior nerves

In conclusion, I would submit that the occurrence in the oculomotor of afferent nerve fibres (conducting centripetally impulses arising in sensorial end-organs), and of ganglion cells upon the root of the nerve almost certainly related to these afferent fibres, taken in conjunction with the part which this nerve plays in the development of the ciliary ganglion, constitutes evidence in favour of the complete segmental character of the nerve too important to be ignored Neal, who upholds a view opposed to this, says

* Schäfer, '00, p. 348.

† Sherrington, '94*a*.

that, in his opinion, "the demonstration of the serial homology of head and trunk metameres depends largely upon the proof of the resemblance of eye-muscle and spinal somatic motor nerves" It seems to me that the demonstration of the resemblance of the oculomotor (and other eye-muscle nerves) to a complete spinal nerve (including sensory as well as motor roots) would have even greater value in establishing the segmental character of the head metameres Thus, while I dissent from Neal's statement just quoted, I am altogether in accord with his further statement concerning the eye-muscle nerves that "failure to convince morphologists of their meristic homology with spinal nerves would tend to undermine the foundations of the traditional conception of the head."

I desire to take this opportunity to acknowledge my indebtedness to Prof. Dendy for valuable advice and criticism, and to Profs Elliot Smith and J. P. Hill for kindly directing my attention to the literature of the subject

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The Osmotic Balance of Skeletal Muscle

By Dorothy Jordan Lloyd

(Communicated by W B Hardy, F R S Received February 24, 1915)

Fletcher was the first to follow continuously, for any considerable length of time, the change in weight of a muscle immersed in a hypotonic solution.* He found that the muscle at first increased in weight and then decreased In isotonic solution the muscle "neither gains nor loses weight." This amounts to a definition of an isotonic solution.

The changes in weight of the gastrocnemius or sartorius, the muscles used by Fletcher, are slow, owing to the low value of the ratio of surface to volume Very early in this work therefore it was decided to use a thin flat muscle sheet The sternocutaneous muscle of the frog was fixed upon It reaches its maximal intake from a hypotonic solution in from 5 to 20 minutes according to the concentration of the solution and the state of the muscle In the case of so small a muscle it is possible that all the fibres are nearly in the same state at the same time This cannot be the case with larger muscles. The central fibres of, for instance, the sartorius may be irritable whilst the external fibres are in water rigor Complex physical and physiological reactions between the fibres must occur and complicate the problem An obvious disadvantage of a muscle with a large surface is the magnitude of the error in weight due to variation in the quantity of moisture adherent to the surface The surface was always dried quickly by filter-paper before weighing, and the smoothness of the curves of variation of

* 'Journ. Physiol.,' vol 30, p. 414 (1904).

weight with time is, I think, sufficient proof that the error was reduced to about 1 per cent. of the total weight

According to the definition of an isotonic solution given above, such a solution does not, strictly speaking, exist. A muscle may remain steady within the limit of error of weighing for periods up to half-an-hour, but sooner or later measurable variations of weight appear In other words the muscle removed from the body is a changing system It is a question whether the apparently steady periods are not really periods of very slow change It is noteworthy that different workers have fixed upon solutions of sodium chloride over such a wide range as from 0·6 to 0·8 per cent as being isotonic with frog's muscle, and the only curve given by Fletcher of a muscle in an isotonic solution shows a steady rise in weight

A muscle simply removed from the body is called by Fletcher a resting muscle The use of the term is inadvisable Such a muscle has suffered a certain amount of mechanical disturbance, and in the process of pithing the frog it has been thrown into tetanus lasting a minute or more, at a time when the circulation of blood is defective. Such a muscle is best indicated by the term untreated muscle

The weight changes characteristic of an untreated muscle in solutions of the sugars biose, dextrose, sucrose, and raffinose between the concentrations zero to 0·27 molecular are shown in fig 1, which shews a curve for

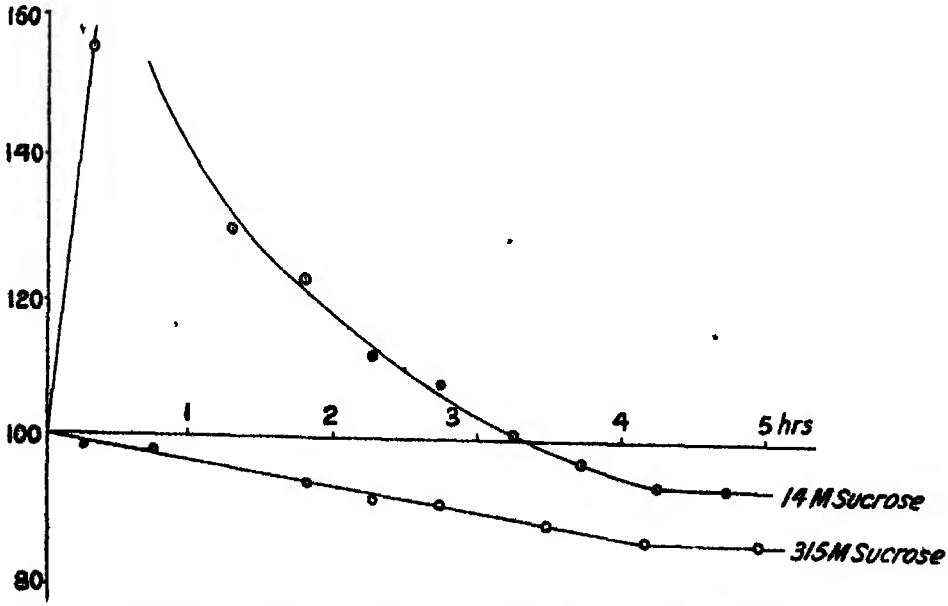

FIG 1.—Abscissæ = hours from beginning of experiment ; Ordinates = weight of muscle expressed in percentage of initial weight.

0 14 molecular sucrose * There is a rapid intake of water followed by loss, the weight often falling much below the initial weight This curve should be compared with the curve of change of weight in hypotonic (0 10 molecular) Ringer, given in fig 2

The curve characteristic of a concentration higher than 0·27 molecular is

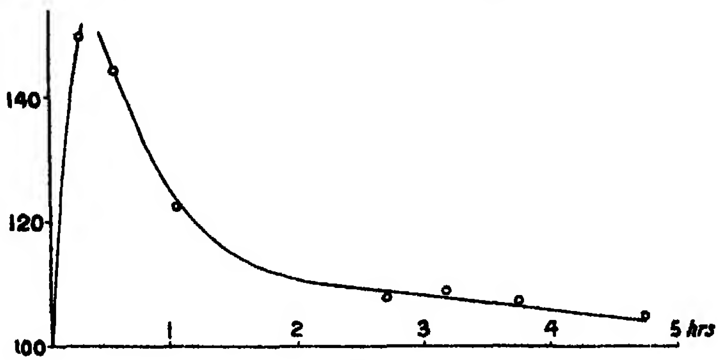

Fig 2

also given in fig 1 The initial intake not only is not present but is not even represented by a variation in the rate of loss The relation throughout is simply linear

The fact that the loss of weight in the more concentrated solutions is in linear relation to time is of interest The osmotic equivalent of an untreated muscle exposed to a solution such that it takes in water, clearly undergoes a change since the intake gives place to a loss in weight usually greater than the previous gain Does the osmotic equivalent of the surviving muscle spontaneously change or is the change just mentioned due to the exposure to a hypotonic solution? The linear form of the curve of loss in a hypertonic solution suggests that the variation of state is due to the influence of the solution The linear form of the curve also would imply that the loss is due to a change in the state of the muscle, for if it were merely the establishment of an osmotic balance with a fixed effective mass of solute within the muscle the rate would diminish as the effective concentration within the muscle approached that outside it. Both above and below a certain concentration the progress of change bears the character, not of the simple establishment of osmotic equilibrium between two solutions initially of different concentration, but of the response of a labile system to an external change of state

* It must be remembered that a 0 125 molecular solution of sodium chloride (taken by most writers as isotonic) is osmotically equivalent to a 0·23 molecular solution of a sugar

The region between 0·21 molecular and 0 27 molecular for the sugars is one in which the initial intake may or may not appear. The muscle either at once loses weight after a short period of slight change, or shows a typical initial intake followed by a typical loss The variation over the region may provisionally be attributed to a variation in the state of the muscle due to mechanical disturbance For instance, in dissecting the muscle out it is subjected to a varying amount of tension, and this will tend to produce passage of fluid from the interior of the fibre to the lymph space or *vice versâ*, and pithing the frog causes fairly prolonged twitching when the blood flow is poor If it were possible to secure muscles in a definite physiological state this diffuse zone would probably narrow to a critical concentration, below which the initial intake would occur and above which it would vanish Some part of the initial intake of water from hypotonic solution is unquestionably due to the mechanical disturbance of the muscle

Effect of Oxygen—Fletcher found that the osmotic changes induced in a muscle by activity were removed by exposure to oxygen. The muscle was put back into the "resting" state, which was characterised by a large intake of water from hypotonic solutions My results with the sterno-cutaneous do not readily harmonise with those of Fletcher The effect of previous exposure to oxygen is to reduce, and finally to obliterate, the initial intake of fluid even from distilled water In fig 3 are two curves, (*a*) from a muscle put directly after removal from the body into distilled water, (*b*) from a muscle placed in distilled water after three hours' exposure to moist oxygen.

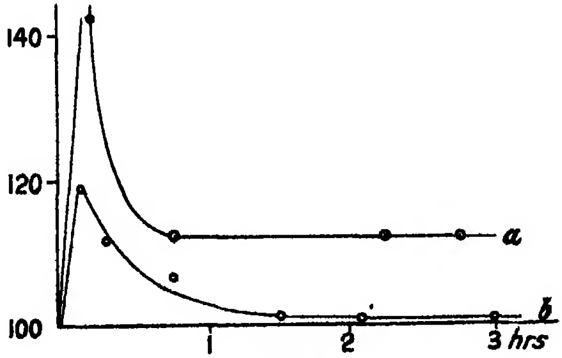

FIG 3.

It might be urged that the intake has already taken place from the water vapour during exposure to oxygen. This is not so Muscles in oxygen and water vapour always tend to lose weight. If any intake does occur it must be so transitory and slight as to have escaped detection

Exposure to Water Vapour.—The behaviour of a muscle immersed in a

solution must always be open to a variety of interpretations. The course of events depends not only upon the initial state of the muscle but also upon properties of the surfaces of muscle and muscle fibre considered as semi-permeable membranes Further, osmotic relations are complicated by change in the muscle due to the chemical nature of the solution Thus, to take salts as examples, the sternocutaneous muscle in $\frac{1}{8}$ molecular solution of sodium chloride usually remains of constant weight for some time, and then loses weight, in a similar solution of potassium chloride there is an immediate and prolonged rise in weight followed by a fall, in an isosmotic solution of calcium chloride there is a very small and fleeting initial rise in weight followed by a long and steady fall which may reduce the muscle to 60 per cent of its original weight

It is possible, however, to examine the osmotic balance of a muscle by exposing it to water vapour of varying pressure The gas space round the muscle then acts as a theoretically perfect semi-permeable membrane so far as non-volatile solutes are concerned The method adopted was to suspend the muscle in a flask over a solution of known concentration, the gas in the flask first having been shaken thoroughly with the solution and then left at the desired temperature for some hours in order to attain equilibrium The muscle was removed for each weighing, and results therefore are affected by an error due to loss of water during weighing, and loss of vapour from the flask during removal and replacement Control experiments with fine plates of agar saturated with water gave a maximal loss of 3 per cent in four hours.

In fig 4 are shown curves of the weight changes of muscles suspended in oxygen above the plane surface of (*b*) distilled water, (*c*) 0·06 molecular

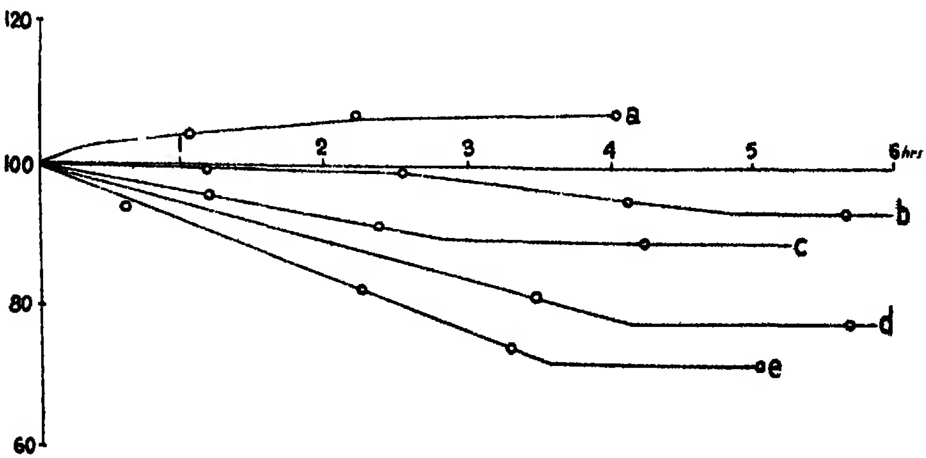

FIG 4

Ringer's fluid, (*d*) 0·125 molecular Ringer's fluid, and (*e*) in air above 0·13 molecular Ringer's fluid, and (*a*) in hydrogen over distilled water.

In oxygen saturated with aqueous vapour over distilled water the weight may either remain constant for a variable period and then fall, or start falling at once. That is to say, oxygen obliterates the intake from vapour which occurs in air or hydrogen. There is here at first sight a contradiction. If the untreated muscle has a vapour pressure less than that of water—as would appear from the curve *b*, fig. 4—why does it not at first condense vapour when in oxygen? Oxygen cannot instantly remove the condition which leads to the intake.

A reason may probably be found in the nature of the diffusion column which must be formed at the free surface of the muscle and of each fibre. At the free surface are water vapour and oxygen. Consider a superficial shell at the surface. If the presence of free oxygen within this shell either raises its vapour pressure to that of water, or maintains its vapour pressure at that level, the quantity of water vapour taken up by the shell will depend upon the ratio of the rate of diffusion of water vapour to that of oxygen. The diffusion column of oxygen progressively retards the diffusion of water vapour, so that, even if their diffusion rates initially were equal, that of the water vapour would rapidly tend to vanish. An analogous retardation is that seen when a retardation in the dissipation of heat or diffusion of impurities from the face of the solid arrests the solidification of over-cooled liquids.*

In hydrogen saturated with water vapour the muscle gains in weight. Strictly speaking, the gas was air very much diluted with hydrogen and saturated with water vapour. In this the sternocutaneous maintains its irritability for about six hours.

What change in the muscle is it which is caused by excess oxygen and which raises the vapour pressure? The work of Fletcher† and of Fletcher and Hopkins‡ suggests that exposure to oxygen reduces the concentration of metabolites (such as, for instance, lactic acid), and so raises the vapour pressure. This would accord with the view of Ranke and of all who have followed him, that the intake of water by a fatigued muscle is due to the production during activity of chemical substances of low molecular weight.

The secondary fall in weight of muscles due to loss of water was ascribed by Fletcher to a "loss of the semi-permeable character of the fibres," just as an ordinary osmometer whose membrane is not completely impermeable

* Wilson, 'Phil. Mag.,' (5), vol. 50, p. 238.

† 'Journ. Phys.,' vols. 23 and 28.

‡ 'Journ. Phys.,' vol. 35, p. 247 (1906–7).

to a solute shows first an intake of solvent and later, as the solute escapes, a loss If this were the sole cause, then the secondary loss of water would be due, either directly or indirectly, to loss of carbonic acid, for this is the only known solute which can escape into the vapour.

It must be pointed out that these experiments cannot be used to calculate the vapour pressure of the muscle substance because of the error in weighing mentioned above, and because the rise of the vapour pressure of the muscle due to curvature of the surfaces is included

Summary

1 The sternocutaneous muscle of the frog, immersed in a hypotonic solution of Ringer's fluid, of biose, dextrose, sucrose, raffinose, or of NaCl undergoes first a gain in weight and later a loss

In a hypertonic solution the weight falls from the start

2 The initial gain in weight in hypotonic solutions or even in distilled water can be reduced and finally suppressed by previously exposing the muscle to wet oxygen

3 Muscles absorb water from an atmosphere of hydrogen and water vapour, but not from one of oxygen and water vapour In the latter a fall in weight was observed

Surface Tension and Ferment Action

By E. Beard and W. Cramer

(Communicated by Sir Edward Schäfer, F R S Received March 17, 1915)

(*From the Physiology Department, Edinburgh University*)

The following investigations were carried out with the object of determining whether the action of a ferment on a substrate is affected by surface tension Since in the living organism the action of ferments proceeds in a system in which surface development has reached a maximum, the problem is one of considerable theoretical importance So far as we are aware, it has not been studied The experimental difficulty is, of course, to allow the factor of surface tension to operate on the action of ferments in such a way that a sufficient amount of the digest can be obtained at the end of the experiment in which the progress of the ferment action could be determined Various devices were used to attain this object In some preliminary experiments the reaction was allowed to proceed in a capillary tube, in others in test-tubes filled with glass wool, with thin short capillary glass tubes or with glass beads The reaction as it proceeded in these tubes was then compared with that of a control in an ordinary test-tube. A distinct effect was observed in these preliminary experiments with lipase, diastase, and yeast invertase, all of which showed a retardation. The effect was then studied in some detail in the case of invertase

The experiments with invertase were carried out as follows —Solutions of sucrose and invertase were mixed in definite proportions Part of the mixture was placed in a test-tube and served as a control The rest was put into test-tubes filled with glass beads, 3 to 4 mm in diameter Care was taken that the level of the fluid was always well below the top level of the glass beads. The tubes were then closed with a rubber stopper and incubated at a given temperature After a given number of hours, readings were taken with a polarimeter. In every series of experiments the control tubes were read first, so that the slightly prolonged period of incubation in the tubes filled with beads would tend to diminish any retardation that might occur. Special control experiments showed that the presence of glass beads did not affect the readings obtained with pure sucrose solutions. Similarly the readings obtained with solution of invertase alone remained constant. That the effect of the mutarotation of the glucose formed by the action of invertase could be neglected will be pointed out below. The beads were washed after each experiment for several hours, first in hot running tap water, then with distilled water, and dried in an oven at about 180° C

The invertase was prepared from yeast obtained from Distillers Co., Ltd., Edinburgh, by two different methods —

(1) *Preparation of Invertase from Fresh Yeast* (called "Invertase F" in the following Tables) —A weighed quantity of yeast was added to a measured volume of chloroform water The mixture was kept at a temperature of 38° for 27 hours or longer. It was then filtered The proteins were removed from the filtrate with kaolin The second filtrate was a clear yellow liquid, which was used as the solution of the ferment

(2) *Preparation of Invertase from Dried Yeast* ("Invertase D" in the following Tables) —Fresh yeast was pounded with distilled water and washed three times by decantation It was then collected in a Buchner funnel, washed with alcohol and ether and spread in a thin layer on a glass plate It was completely dried in a vacuum desiccator. When dried the material was ground in a mortar, placed in an oven at 40° and gradually warmed during half an hour up to 100° The brown powder then obtained was kept in a stoppered bottle When a solution of invertase was required, weighed amounts of the brown powder were added to chloroform water and kept for about 24 hours at a temperature of 38° After filtration a clear yellow liquid was obtained, which represented a solution of invertase

Eight different invertase preparations, all of which were strongly active, were used in these experiments In the Tables the various ferment preparations are designated (1) by the letter D or F, indicating whether they have been prepared from dried or fresh yeast respectively, (2) by the date of their preparation, and (3) by the percentage of yeast (fresh or dried) used with reference to chloroform water

It was found that increasing the surface led to a distinct retardation in the inversion of cane sugar by invertase if the concentration of the substrate was relatively high, and that of the ferment relatively low With a high ferment concentration the retarding effect was not noticeable. The following experiments are given as examples :—

Experiment A 23 7.14. *Invertase F*, 22.7.14, 100 *per cent*
Sucrose solution 19 6 per cent. Temperature 18°. 50 c.c. sucrose :

Hours	+5 c c. invertase		+1 c.c invertase		+0·2 c c invertase.	
	Control	With beads	Control.	With beads.	Control.	With beads.
0	+11·88	+11·88	+12·93	+12·93	+13·02	+13·02
22	− 3·21	− 3·21	+ 4·93	+ 5·13	+11·06	+11·76
47			− 0·25	− 0·03	+ 9·03	+ 9·71
96			− 3·16	− 3·08	+ 5·80	+ 7·09

Since in all the following experiments relatively small concentrations of invertase were used, so that the process of inversion was slow, the effect of the mutarotation of glucose did not affect the readings This was verified by special control experiments in which dilute sodium carbonate was added to the solution before the readings were taken.

The retarding effect of surface tension was confirmed in a number of other experiments It is not necessary to quote these experiments in detail, as the effect is evident in the experiments given below which deal with the further analysis of the phenomenon

In the action of an enzyme on a substrate one may distinguish two separate phases firstly, the combination of the enzyme and the substrate, and, secondly, the chemical change which proceeds in this compound of enzyme and substrate. *A priori* surface tension may have an effect either on the first phase or on the second phase or on both

We will consider first the possibility of surface tension action on the second phase of enzyme action. Just as the catalytic action of an enzyme has been likened to a lubricant producing its action by diminishing friction, so the effect of surface tension might be like that of a brake, retarding the process by increasing the friction If that were so, one would expect to find that retardation disappears when the brake is removed, *i e* when surface energy is reduced to the dimensions which obtain in the control To test this point a number of test-tubes filled with beads were charged with the mixture of substrate and ferment After a given number of hours, when a distinct inhibition had become noticeable, the fluid (or part of it) was removed from one of the test-tubes filled with beads, and inversion allowed to proceed as usual in a test-tube without beads An example will make the arrangement clear.

Experiment B 22 6 14 *Invertase D*, 18 5 17, 10 *per cent*

2·5 c c. invertase and 100 c.c sucrose, 20 per cent. Temperature of digestion 27°

Hours	Without Beads				With Beads			
	Control tube	Tube 1A	Tube 2A.	Tube 3A	Tube 1	Tube 2	Tube 3	Tube 4.
0	+12·90				+12·90	+12 90	+12 90	+12·90
10	+12·14				+12 59	—	—	—
24	+11 37	+11 80				+11 88	—	—
48	+9 26	+10·25	+10 42				+10 57	—
96	+5·60	+7·66	+7·75	+8·08				+8 47

In this particular experiment the inhibition disappeared only partially after reducing the surface energy. It is still evident after the removal of beads,

but not so marked as in the test-tubes in which the surface remained extended Similar results were obtained in a number of other experiments, with other preparations of invertase

A very different result was obtained, however, in the following experiment carried out with the same preparation of invertase but at a higher temperature and with a slightly lower ferment concentration

Experiment C 15 6 14 *Invertase D*, 18 5 14, 10 *per cent*
1 c c invertase and 100 c c sucrose solution, 19·5 per cent
Temperature 42°

Hours	Without beads: Control tube	Without beads: Tube 1A	With beads: Tube 1	With beads: Tube 2
0	+12·63		+12·63	+12·63
18	—		+12 47	—
43	+10 12		+12 48	+12 45
66	+ 8·93	+12 22		+12·29
90	+ 7 80	+12·09		+12 30

Here the inhibition in the system in which the surface has been extended retains its full strength after the surface has again been reduced to the dimensions of the control

The opposite condition was realised in only one experiment

Experiment D. 20 5 14 *Invertase F*, 23 4 14, 25 *per cent*
10 c c invertase and 100 c c sucrose, 9 8 per cent. Temperature 18°.

Hours	Without beads: Control	Without beads: Tube 1A	With beads: Tube 1
0	+5·77		+5·77
13	+4 44		+4·93
17	+3·96		+4 71
37	+2 12		+3 40
43	+1·62	+3·03	+3·25
48	+1·21	+2·67	—
64	+0 32	+1·89	+2·47

Here the inhibition is almost completely removed when surface energy is reduced.

The conclusion to be drawn from these experiments is that the retarding action of surface tension on the inversion of cane sugar by invertase is made up of two components. One component leaves the system cane sugar-invertase

unchanged when surface tension ceases to operate. The action of this component would be in accordance with the first alternative suggested above, namely, that surface tension acts as a brake on the chemical process which proceeds in the substrate under the influence of the ferment.

The action of the second component involves a permanent alteration in the system cane sugar-invertase. How is the action of this component to be interpreted?

We have considered hitherto only the possibility that surface tension inhibits the second phase of an enzyme action, namely, the chemical change which proceeds in the substrate after it has become combined with the enzyme We may now examine what effect surface tension could have on the first phase of an enzyme action—the combination of enzyme and substrate. The question is then · Is it *a priori* possible that surface tension could inhibit the combination of enzyme and substrate, and if so, how can this possibly be tested experimentally?

It is a well known fact that ferments tend to go into the surface layer, so that the concentration of the ferments is higher on the surface layer than in the remainder of the solution This property of ferments is expressed by the statement that ferments are "surface active" By increasing the surface, therefore, more ferment will be driven into the surface layer Cane sugar, on the other hand, is practically not surface active, and its distribution in the solution will, therefore, remain unaltered when the surface is increased Theoretically, therefore, it would seem possible that by extending the surface a certain amount of invertase is driven into the surface and thus prevented from combining with the cane sugar

The point is capable of being tested experimentally If new surfaces are created the ferment is, as just stated, driven into the surface layer If, for instance, a ferment solution is shaken so that foam is formed, the concentration of the ferment in the foam is greater than that in the rest of the solution If now the foam is allowed to subside, the concentration of the ferment in the solution rises again, although the original value may not be reached. But if new surfaces are created by the introduction of solid substances, a second phenomenon may come into appearance The ferment not only goes into the surface layer bounding the solid, but may become adherent to the solid, so that when the new surfaces are destroyed by withdrawing the solid, the ferment remains adherent to the solid and is withdrawn with it.

This is the phenomenon of adsorption, which is exhibited in a marked degree by a number of ferments brought into contact with solids such as charcoal, collodion membranes, suspensions of mastix. It is not necessary to

discuss here the conditions on which adsorption depends. In connection with the present problem the phenomenon of adsorption is of interest only in so far as it presupposes as a preliminary condition a concentration of the adsorbed material at the surface of the adsorbing material. It must also be borne in mind that such a surface concentration may occur without adsorption taking place, as in the case of foam, for instance, where the surface separates a gas and not a solid from the liquid. We shall, therefore, use the general term of "surface concentration" to describe the alteration of concentration produced in a system by altering the surface energy, and the term "adsorption" in order to designate the special result which is caused under certain conditions by surface concentration If it can be shown then that under the conditions of our experiments invertase is adsorbed by glass beads, we would have evidence that the factor of surface concentration accounts, at any rate partially, for the retardation of the ferment action observed in our experiments If, on the other hand, no adsorption is observed, no conclusions can be drawn either for or against this possibility, as one may conceive of surface concentration occurring without adsorption

To test this point, experiments were carried out, in which invertase was kept in contact with glass beads. Another portion of the same ferment preparation was allowed to stand in a test-tube by itself at the same temperature as a control After a definite number of hours the ferment solution was removed from the glass beads and its activity compared with that of the control The former preparation will be described in the Tables as "contact invertase," the latter preparation as "control invertase"

The following experiments may be given as examples of the results obtained :—

Experiment E 14 5 14. *Invertase D*, 6 5 14, 5 *per cent*

Kept for 93 hours at 38°, (1) in contact with beads; (2) alone as control. 5 c c of each of these two preparations mixed with 5 c.c sucrose solution, 5 per cent Digested at 38°.

Hours.	Contact invertase	Control invertase.
0	+3·26	+3·25
5	+3 25	+2·28
27	+1·98	−0 86

(Note that in this experiment the relative amount of ferment used for inversion is very large.)

Experiment F 16.5.14. *Invertase Preparation same as in Previous Experiment*

Kept for 25 hours at 41°, (1) in contact with beads, (2) alone as control. 1 c.c of each added to 10 c c sucrose solution, 9 6 per cent. Digested at 18°.

Hours	Contact invertase	Control invertase
0	+5·79	+5·70
19	+2·92	+1·99
42	+0 44	−0 52

It will be noted that there is a marked effect in both cases, and that with the longer exposure of the ferment to the beads, the disappearance of the ferment becomes more marked.

The effect of temperature will be seen from the following experiment —

Experiment G 7.7 14 *Invertase D,* 30.6 14.

Kept for 22 hours, (1) in contact with beads at 18°, 29°, and 40°, and (2) alone as control at the same temperature 1 c.c of each of the six ferment solutions added to 10 c c. of sucrose solution, 9 per cent Digested at 30°

Hours	18°		29°		40°	
	Contact invertase	Control	Contact invertase	Control	Contact invertase	Control
0	+5 99	+5 99	+5·99	+5·99	+5 99	+5 99
26½	+2·78	+1 79	+2·37*	+1·67*	+4 44†	+3·74†

* Examined half an hour later than tubes kept at 18°

† Examined one hour later than tubes kept at 18°

This experiment is of interest because it shows, in addition to the disappearance of invertase owing to adsorption, a destruction of the ferment even in the control at higher temperatures (40°). This destructive effect is apparently enhanced by increasing the surface. This effect would explain the results obtained in Experiment C (15.6 14), where there was not only an almost complete inhibition of the action of the ferment, but where this inhibition persisted even after the factor of surface energy was again removed.

The experiments just quoted, which were carried out with two different preparations of invertase, gave a distinctly positive result with reference to

adsorption With two other preparations no evidence of adsorption was obtained The following experiment is given as an example :—

Experiment H. 20.5.14. *Invertase F*, 23.4.14, 25 *per cent.*

Kept for 20 hours at room temperature, (1) in contact with beads, and (2) alone as control 1 c.c. of each added to 10 c.c. sucrose solution, 10 per cent. Digested at room temperature.

Hours	Contact invertase	Control
0	+5·77	+5·77
18	+3·98	+3·84
23	+3·53	+3·35
29	+2·91	+2·79
45	+1·76	+1·68
90	−0·31	−0·36

This experiment should be compared with the results obtained in Experiment D, in which the same preparation of invertase was used In this experiment the inhibition produced by increasing the surface disappeared again when the surface was reduced to the dimensions of the control.

The same absence of adsorption was observed with a second preparation of invertase This preparation showed the usual inhibition of its action by surface tension, and as in the previous case this inhibition disappeared in two experiments when the factor of surface tension was removed. In a third such experiment in which less invertase was used the inhibition persisted partially

General Remarks

The observations demonstrate that the action of invertase on cane sugar is retarded by increasing the surface of the system. They show further that this retardation is due partly to a surface concentration effect: the surface-active ferment is driven into the surface and thus prevented from combining with the surface-inactive cane sugar If one looks upon the combination of substrate and enzyme as a surface concentration effect, as Bayliss does, one can readily understand that this combination can be inhibited by the same force acting in the opposite direction, so that these observations form incidentally a confirmation of the conception formulated by Bayliss.

A question which cannot yet be answered with certainty is whether the retardation observed in these experiments can be explained entirely as a surface concentration effect, or whether surface tension acts also by retarding the chemical process taking place in the substrate, that is, the second phase of ferment action. That surface tension retards certain chemical processes, for

instance, the formation of chloroform from chloral by alkali, is a well known fact. In the present case it has been found that the action of invertase on cane sugar may be retarded even although the effect of adsorption is not noticeable under the conditions of the experiment. It has also been found that in some cases the retardation disappears almost completely when the influence of surface tension is removed after it has been operative. Observations such as these would be most readily explained by assuming that the second phase of ferment action has been inhibited by surface tension and not the first phase. But even when there is no evidence of adsorption we cannot exclude the possibility of surface concentration occurring without adsorption In other words it seems possible that the ferment goes into the surface layer of the liquid without becoming adherent to the solid and without being removed with latter Such a condition would also explain the observations referred to above

We have convinced ourselves by a number of preliminary experiments that the retardation by surface tension is a phenomenon shown by other ferments besides invertase The conditions in the case of other ferments have not been studied by us in detail. But it is evident that the effect obtained may differ with the nature of the ferment, of the substrate, of the products of ferment action, and of the surface If for instance the substrate itself is surface active, the conditions will differ markedly from those which obtain when the substrate is surface inactive

Surface Tension as a Factor Controlling Cell Metabolism.

By W Cramer.

(Communicated by Sir Edward Schäfer, F.R.S Received March 17, 1915.)

Although it is known that many chemical processes taking place within the cell are due to the actions of ferments, and although we can in many cases separate these ferments from living protoplasm and study their action *in vitro*, there still remain considerable discrepancies between the processes as we see them occur *in vivo* and as we study them *in vitro* One of the most characteristic features of the processes taking place within the living cell is what, for want of a better term, may be called their "adaptability," that is the delicate sensitiveness with which they respond to very slight changes in the surrounding medium by being retarded, accelerated, or reversed It is known, of course, that the action of ferments is influenced by changes in temperature or in the alkalinity or acidity of the surrounding medium But in the case of the living cell these factors remain practically constant, so that their influence can be excluded We know, too, that many reactions brought about by the action of ferments are reversible, and that the direction in which the ferments act depends upon the concentration of the various substances entering into the reaction But here again these differences are of an order of magnitude far greater than the variations which exist in the living organism It is noteworthy, too, that the equilibrium of a reaction brought about by a ferment separated from living protoplasm lies almost always near the point of complete hydrolysis, and contrasts in that respect markedly with the behaviour of the same ferment when its reaction is studied in the living cell, where the reverse process may be found to occur or where very minute changes in the surrounding medium are sufficient to transfer the point of equilibrium from hydrolysis to synthesis

If we take the liver cell as an example we find that the living cell can, with equal readiness, transform glycogen into glucose and glucose into glycogen If the liver is removed from the body a marked glycogenolysis occurs, so that a marked amount of sugar is formed, while the power to synthesise glycogen appears to be almost completely inhibited. In other words, the equilibrium point lies now near the point of complete hydrolysis. The same takes place if an extract of the liver is allowed to act on glycogen or on glucose in the concentrations found in the blood. It is known that the formation of glycogen *in vivo* occurs when the percentage of blood-sugar is

relatively high, and the reverse process when the blood-sugar concentration falls. But the differences in the concentration of the blood-sugar which accompany these processes are too slight to be an adequate explanation in themselves, especially if we compare them with the large difference of concentration necessary to effect the reversal of a ferment action *in vitro*. Moreover, when the liver is removed from the body the formation of such a large amount of sugar takes place that the hydrolysis of glycogen should be inhibited if the concentration of the sugar was the main factor. Nevertheless the glycogen under these conditions completely disappears. The disappearance of glycogen from the liver in the living animal as the result of "sugar puncture" or under the influence of the thyroid hormone cannot be satisfactorily explained on the ground of changes in concentrations of the reacting substances.

In order to explain the predominance *in vivo* of the synthetic power of ferments which *in vitro* act almost entirely as hydrolytic agents, the assumption has been made that *in vivo* the products of synthetic action by a ferment are withdrawn, as they are formed, from the sphere of action, so that the equilibrium is always being disturbed in favour of the synthetic process. If we take the liver again as an example, we find that with the ferment acting *in vitro* the equilibrium point lies near the point of complete hydrolysis. That means that a very small amount of glycogen can be synthesised by the ferment, even *in vitro*. But since the glycogen thus formed remains in solution the reaction stops when once this point of equilibrium is reached. In the cell the slight amount of glycogen synthesised by the ferment is deposited in an insoluble form as it is formed. The equilibrium is thus disturbed and another slight amount of glycogen is formed. In other cases it is assumed that the product of synthesis is removed by diffusion or excretion or carried away by the tissue fluids.

Now this consideration may account for the fact that ferments which show only a slight synthetic power *in vitro* have a marked synthetic action *in vivo*. It is doubtful whether this explanation can be applied in every case. It certainly does not explain the "adaptability" of the cell, the readiness with which the cell metabolism responds to slight changes in the environment. It does not explain, for instance, the ease with which the liver cell regulates its glycogenic function in the one or other direction, why a difference of less than 0·1 per cent. in the concentration of the blood-sugar determines whether synthesis or hydrolysis of glycogen is to take place, or why the "piqure" and the thyroid hormone produce a "mobilisation" of glycogen.

It is clear, therefore, that there are factors conditioning the actions of ferments within the cell which do not come into play when we study the

actions of these ferments *in vitro* under the usual conditions, and which have therefore escaped observation.

If one compares the conditions under which chemical processes proceed *in vivo* with the experimental conditions under which the same processes are usually studied *in vitro*, one finds as the most obvious difference that in the latter case the influence of surface energy is reduced to a minimum, while in the former it is developed to a maximum. *In vivo* there are interfaces between cytoplasm and the surrounding medium, cytoplasm and nucleus, cytoplasm and deposits in the cytoplasm, besides the interfaces presented by the various colloids constituting the cytoplasm. In the conditions usually obtaining in experiments *in vitro* all these sources of surface energy are non-existent, only the colloidal nature of ferment or substrate may give rise to surface-tension effects, as they do, of course, also within the cell.

The question is therefore whether surface-tension effects (apart from those possibly caused by the colloidal nature of ferment or substrate) are factors conditioning the action of ferments It was found that when this factor of surface tension was introduced by allowing the reaction to proceed in a test-tube filled with glass beads or in a capillary glass tube, so that the surface was increased, the action of invertase, diastase, and lipase was distinctly retarded.

A detailed account of the results observed with invertase is given in the preceding paper.

Further analysis of the phenomenon showed that the two phases which can be distinguished in the action of a ferment are probably both subject to the influence of surface tension, firstly the combination of substrate and ferment, and secondly the chemical reaction which takes place in the substrate and in which the ferment acts as a catalyst.

While these observations establish the principle that ferment action is conditioned by surface tension, they can only give a faint and incomplete idea of the degree to which this factor controls the action of ferments within the cell For, compared with the immense development of surface which obtains in the living cell and the living organism, the increase in surface energy produced by the presence of glass beads in the mixture of ferment and substrate is very small. It must also be borne in mind that the experimental conditions deal with surface tension between glass and a watery solution, while *in vivo* surface-tension effects are produced between colloidal solutions of different composition, membranes, colloids in the form of gels, and so forth. Lastly, the effect of surface tension may vary with the nature of the ferment, of the substrate and of the products of ferment action, according to the "surface activity" of these substances, *i.e.* their property to lower surface tension.

The first general conclusion which may be drawn from these considerations is that the great surface development in the cell or the organism produces conditions which markedly affect the action of ferments *in vivo* when compared with their action *in vitro*

We may now consider in some detail in what way this surface development exercises its effect with regard to the metabolism of the cell. We find then, firstly, that different parts of the cell present different conditions for the action of ferments Surface tension is operative at the periphery of the cell, at the interface between cell and the surrounding medium There the conditions for ferment action will differ from the conditions presented by the interior of the cell But even in the interior of the cell, conditions in the protoplasm surrounding the nucleus, vacuoles, granules, etc, will differ from those presented by the rest of the cytoplasm We must then conclude that ferment action will not proceed evenly throughout the cell It may be retarded or inhibited in one part of the cell, while it is proceeding actively in another Whether it can even be reversed as the result of surface tension is not yet clear from the experimental evidence before us In this connection reference may be made to the work of Warburg, who has demonstrated that the oxidative processes in the cell are dependent on the structural parts of the cell and not on the fluid cell contents

We have hitherto assumed, for the sake of simplicity, that the surface tension of protoplasm is constant But we know that it is always changing as the result of chemical processes leading to the formation or disappearance of surface-active substances In cells with free surfaces, such as unicellular organisms for instance, these fluctuations in surface tension result in the decrease or increase of surface: they become manifest in the form of movement. Amœboid movement and ciliary movement have long been recognised as surface-tension effects The existence of such changes in different physiological conditions of a unicellular organism has also been demonstrated recently by MacCallum, by a micro-chemical study of the distribution of potassium salts within the cell

Similar changes must occur also in cells aggregated in cell masses or organs But here, where the surfaces are not free, and possibly less elastic, and where the cell cannot extend or diminish its surface except to a limited extent, the result is, not movement, but alterations in the concentration and composition of the substances constituting the surface layer of the cell, and this leads to further alterations of the cell metabolism.

Thus surface tension conditions cell metabolism, and is, in turn, also conditioned by the metabolism of the cell. Chemical changes within the cytoplasm may lead to the formation or disappearance of surface-active substances

or to variations in their concentrations. And it may be pointed out here that surface tension is affected by very slight quantities or changes in concentration of surface-active substances, particularly when the initial concentration is low. Or interfaces may be formed, or disappear, as the result of chemical changes, or become extended or shortened, and thus affect again chemical changes, just as in the experiments with invertase the reaction was influenced by extending the surface "glass" through the watery solution We thus get a conception how the cell regulates and controls its metabolism, how a chemical change may accelerate or retard other chemical processes which may have no chemical relation to it and which *in vitro* would remain unaffected by it It is thus possible to explain the chemical organisation of the cell without having to postulate, as has hitherto been done (Hofmeister, Hans Meyer), the existence of hypothetical membranes in the cytoplasm which are supposed to separate the different chemical systems

The validity of this conception of surface tension and surface energy as factors controlling cell metabolism is capable of being tested experimentally. For, according to this idea, substances which do not affect the protoplasm chemically (that is to say are neither bases nor acids, neither reducing nor oxidising agents, etc), but which are strongly surface active, should markedly affect the metabolism of the cell. That is actually the case The condition of narcosis in which the metabolism of the cell is profoundly affected is brought about by substances which fulfil these postulates. They do not affect the protoplasm chemically and they are all strongly surface-active; there is even direct evidence that these substances influence the action of cell ferments. For Chiari has shown that in the excised liver of anæsthetised animals the process of autolysis proceeds more rapidly than in the liver of normal animals. And Guignard has demonstrated that the effect of anæsthetics on laurel leaves is to release the action of ferments, as evidenced by the production of hydrocyanic acid.*

* In order to avoid misunderstandings, it may be pointed out here that these substances, in addition, must be capable of penetrating into the cell. They do so by virtue of their solubility in lipoids, according to the Meyer-Overton theory, or by virtue of their "Haftdruck," according to J. Traube. These theories of narcosis enable us to understand quantitative differences in the strength of different narcotics or in their action on different tissues. But they do not enable us to understand how they act upon cell metabolism in such a way as to bring about the state of narcosis when they have penetrated into the cell, and it is this question which is being considered in this paper.

Hans Meyer himself recognised this, and put forward as a further explanation the assumption that narcotics soften the hypothetical lipoid membranes which surround each molecule of ferment and substrate and keep them apart under normal conditions. Others (Höber, Mansfeld, Gürber) have put forward different hypotheses to explain the state of narcosis.

We arrive at a similar confirmation of the relation of surface-active substances to cell metabolism, when we examine the substances which, as Loeb has shown, affect the metabolism of unfertilised eggs in such a way that they either produce cytolysis or, under certain conditions, incite artificial parthenogenesis The substances which belong to this group—narcotics, saponin, soap, bile salts—are all substances which are strongly surface active

OBITUARY NOTICE

OF

FELLOW DECEASED.

CONTENTS.

ARTHUR LISTER, 1830–1908

My father was the youngest of four sons of Joseph Jackson Lister, F.R.S. The second was Joseph, afterwards Lord Lister, the founder of the modern system of surgery, and past President of the Royal Society. There were three daughters who all married and had children.

The family had belonged on both sides, and for several generations, to the Society of Friends. His mother was of Irish extraction, the daughter of Anthony Harris, a sea captain, whose home was at Maryport, Cumberland, and who owned and sailed his ship; she had before her marriage been a teacher at the Friends' School at Ackworth. I can remember her as a dear, dignified old lady, dressed in the subdued, harmonious colours of the Quaker garb, and muslin cap of the proper cut. I think I remember (I have certainly been told of) her taking exception to some full "bell sleeves" of one of my sisters as "superfluities."

My grandfather I clearly remember as a benevolent-looking, handsome old gentleman, clean-shaven except for short side whiskers. He was very active, even in old age, and I can recall him in his Quaker coat, with the collar not turned down, running backwards on the lawn of his garden while I, a small boy, vainly endeavoured with my utmost efforts to overtake him. He had entered his father's business of wine merchant in London, and quite early in life found himself in a position of comparative affluence. His family was brought up and he remained living, till his death, at Upton House, in the parish of West Ham, then a rural suburb of London on the skirts of Hainault Forest.

It was at Upton that my father was born. There was a beautiful and large garden attached to the house, with two very fine cedar trees beyond the lawn. He employed part of his leisure with investigations on the optical properties of different kinds of glass, and on combinations of lenses, which he ground himself. This led to his discovery of the true principle on which compound lenses should be constructed—an important step in the great modern advance in microscopy. This discovery brought him into touch with several of the foremost scientific men, both English and French, of the day. Prof. Owen was a frequent visitor, and delivered acceptable drawing-room discourses on matters zoological. I remember being introduced to the great man in later years and the kindly interest he took in me as the grandson and namesake of his old friend. Sir John Herschel and Edward Forbes were also friends of the family. The meetings of the British Association, then in its infancy, were frequently attended.

So there was the breath of a larger and cultivated world in the environment of the young people as they grew up, in addition to the strict religious atmosphere which their parents, at any rate in matters of conduct and

Friendly tradition, were careful to maintain. My impression is that less stress was laid on doctrine, and I believe that my grandfather had, at the time of the expected invasion of England by Napoleon, gone through some training as a volunteer

From his early boyhood and throughout his life, my father was an enthusiastic ornithologist. As a boy he used to wrap flannel round his shoes and steal silently about the garden shrubberies, watching the birds and learning the characteristic notes of the species. He pored over White's 'Selborne' and Bewick's 'British Birds,' and essayed himself to make woodcuts copied from the well-known beautiful illustrations of the latter work He used to recall the thrill of pleasure with which he received from his father a set of proper engraving tools and some blocks of boxwood for this purpose Some of his copies of woodcuts are quite remarkably excellent for a boy of 13 In later life, and until his hearing began to grow dull, my father's power of recognising birds by their notes was most exceptional On country rambles with him one knew that, whatever topic was to the fore, and however interested he might be in it, part of his mind was ever keenly on the alert to the doings and songs of the birds He would suddenly stop, listening, and then he might resume his walk with the words, "Perhaps only a yellow-hammer." But generally if he stopped there was something of interest. "A cirl-bunting," he would exclaim, "a snipe drumming," or "a grasshopper warbler"—or what not?—and at once the telescopes of the party would be turned to see the bird, if possible; though verification by sight was rarely necessary if he had heard the note distinctly

He followed his brothers to the Friends' School at Hitchin, kept by Isaac Brown, who, himself something of a naturalist, encouraged his taste for birds and started him on a collection of mosses. I think it was from him that he and his brother Joseph learnt to repeat Latin verses, in the old pronunciation of course, and with a majestic rhythm which I have never heard equalled in the new.

From Hitchin he went to the Friends' School at Grove House, Tottenham, but it was not long before (at the age of 16) he was removed from school, and according to the Friends' custom in those days, when University degrees were not open to them, was "put into business." He was apprenticed to a firm of manufacturing chemists. With them he learnt what he might, both at their London place of business and also, what was much more to my father's taste, at their nursery grounds at Ampthill, Bedfordshire. But, his apprenticeship ended, he was placed by his father as partner in a wool merchant's firm in Bradford—filling a vacancy caused by the retirement of William Edward Forster, afterwards Member for Bradford and Chief Secretary for Ireland. While working at Bradford he had bachelor lodgings at Baildon and keenly enjoyed his wild rambles over the moors. While here he read much poetry and other literature. He had a good memory for poetry and knew a great deal by heart. Milton's sonnets and shorter poems were favourites, and many of the poems of Burns, Shelley, and Moore. He knew

his Shakespeare well, and delighted in Wordsworth He often repeated the lines, and they touch the keynote of his life, from the poem on Tintern Abbey, beginning —

> "And this prayer I make,
> Knowing that Nature never did betray
> The heart that loved her, 'tis her privilege,
> Through all the years of this our life,
> To lead from joy to joy "

The "Ode to Duty" (in part), the "Happy Warrior" and the "Lesser Celandine" were also great favourites. Many of the Odes of Horace and some passages of Virgil he had by heart, and loved to repeat His ear for music was very correct, and he had great enjoyment in it when it was well played While at Bradford he learnt to play the flute, with fine feeling, though he would very rarely, and in later life never, indulge his family by playing He also took lessons in drawing and painting from a most excellent teacher, Mr James Lobley, whose instructions to a drawing school at Bradford received the warm commendation of Mr. Ruskin. From him also his elder children and other members of our circle received lessons of lasting benefit

My father had a high appreciation of pictorial art. Frederick Walker's pictures and some of Millais', in his Pre-Raphaelite stage, received perhaps his warmest admiration In his earlier manhood he practised the gentle art of sketching from nature in water-colour with much success, and assiduously trained his elder children in it, being always most kindly appreciative of their efforts Fidelity to the thing as you see it was the end to be aimed at, and any departure in the direction of an ideal rendering received scant encouragement. As his children attained, in some cases, some higher degree of proficiency, however, his own efforts ceased

At Bradford, too, he first made the acquaintance of Susanna Tindall, daughter of William Tindall, of East Dulwich, who, in 1855, became his wife He soon (in 1857) resigned his place in the Bradford firm and succeeded his father, then retiring from business, in the firm of Lister and Beck, wine merchants, at 5, Tokenhouse Yard, London. He was a representative of the fourth generation of his family in this firm The young family settled at Sycamore House, Leytonstone, on the border of Epping Forest, and within easy reach of his father's house at Upton, and the homes of two of his married sisters.

He soon made himself master of his business, and his advice and opinion were highly valued among wine shippers and merchants.

He was at this time much devoted to shooting and fishing. He was an excellent shot and was a welcome member of shooting parties of his friends. But the shooting he liked best was a long ramble with a friend and dogs, but without beaters, over some wild tract of country, with a mixed bag as the result. He and his brother Joseph, then absorbed in his early observations and experiments on physiology and surgery, often arranged

to spend part of their summer holidays together at some remote fishing resort But he was keenly interested in all his brother's work, and his experiments, cases, and improved methods of treatment were described, discussed, and eagerly canvassed, step by step.

In the summer of 1866 my uncle Joseph and his wife joined my father's family for a summer holiday at Torquay My father then first entered on the study of Systematic Botany with his brother's assistance, he having laid a very good foundation under Lindley, Professor at University College, in the course of his medical training

From this time onward sport passed more and more into the background, and was soon entirely given up, though to the end of his life he loved to handle his guns

From flowering plants my father passed on and resumed his study of British mosses, making exquisite water-colour drawings of them under the microscope with the aid of the camera lucida The use of the camera lucida in microscopic drawing he habitually practised in all his work Each drawing had the magnification indicated, so that the exact size of the structures shown could be easily ascertained Even a rough sketch made in this manner, with the outlines hastily traced, may be of permanent value as a record of the size, shape, and relative position of the parts displayed His own drawings so made, with bold, clear outlines, and the tints and light and shade indicated in washes of water-colour, are models of lucid illustration. He regarded this as a great and too much neglected aid in botanical and zoological departments of study With a half-whimsical perversity he would often make the most beautiful drawing on some scrap of lined paper, and it was then trimmed into an odd polygonal shape, to save space, and gummed into his note-book A clean sheet of drawing paper was regarded as something so sacred that only the more elaborate illustrations had a chance of being fairly displayed It was only by much scolding that his daughters, as they grew up, succeeded in effecting a partial reform.

In this manner, as he extended his investigations to the study of lichens and then to moulds and other fungi, he accumulated a store of accurate and beautifully illustrated notes which were of immense value to him in his work

In studying the specific characters of fungi his power of accurate drawing was of especial value to him, because of the evanescent character of this class of plants and the difficulty of preserving them He also invented a simple and most effective method of recording the characters of the gills and the colour of the spores of the Hymenomycetes. The pileus is cut off at the summit of the stalk and laid overnight on a clean piece of blotting paper with the gills downwards, being covered with an inverted wine glass or other cover. In the case of dark-spored fungi the blotting paper used is white, while for white-spored species a tinted paper is employed The following morning the spores which have been produced in multitudes during the night and fallen in the still air, directly downwards, are found to have defined with exquisite

precision the arrangement of the gills. A wash of gum and water on the back of the blotting paper sets the spores, and a beautiful self-recorded "sporograph," or picture in its own spores, of the under surface of the pileus is obtained.

His brother and his wife came each year from Scotland to spend their Christmas holiday at Leytonstone or, later, at Lyme. Much time would be given, if frost permitted, to skating, which they both keenly enjoyed, or to forest walks, in the company of the children and their cousins But all the notes and drawings of the work in hand would be shown, room would be made at a table for a second microscope (which had been their father's and was always referred to as "Augustus") and the two brothers would pursue the investigations together, often even skipping about the room in their whole-hearted joy at the unfolding revelations

About the year 1879, when working at lichens, and desiring to read Stahl's important papers on their strange double nature, he set himself, with his eldest daughter's assistance, to learn German This was entered on with characteristic vigour, his brother gladly participating, when on his holiday visits. Several of the poems of Goethe and Schiller were committed to memory and repeated with great enjoyment

My father's friend, Dr D H Scott ("dear Scott" as he used to call him), has borne testimony to the thoroughness of his work at lichens During a visit to Leytonstone he says, "The conversation turned on the question of the fertilisation in lichens, as described by Stahl, on whose conclusions some doubt had at that time been cast by the school of Brefeld It then turned out that Mr Lister had fully investigated the subject for himself, he showed the writer a series of drawings of the reproductive processes in *Collema*, which went far to substantiate Stahl's views, since strongly confirmed by the work of Baur and Darbishire." *

While his nephews and elder son were in their boyhood my father was an enthusiastic collector of British Lepidoptera, and delightful evenings were spent in sugaring the trees in his own garden and in Epping Forest This was done in part to encourage a love of natural history in the boys, but largely also from his own love of these exquisite products of Nature's workmanship His other excursions on the zoological side of the border, apart from the Mycetozoa, were mainly concerned with such animals as he met in the course of his microscopical work There are, however, accurate drawings of his of the ascidian *Perophora listeri* found washed up in a storm on the beach at Lyme. He was particularly interested in this species because it had been one of the objects investigated by his father, after whom it was named. With one of the new lenses constructed by himself he had been the first to observe the remarkable reversal in the direction of the heart beats now known to be characteristic of ascidians †

When my parents settled at Leytonstone it was still a comparatively

* 'Journal of Botany,' October, 1908, p. 333

† 'Phil Trans,' 1834, p 365

rural neighbourhood Now greater London has partially engulfed it, though the boundary of Epping Forest happily bars its further advance in this quarter A pleasant garden and a field for cows were attached to the house. To accommodate the growing family additions were made to the house, mainly from my father's own designs, and an agreeable set of rooms was arranged, two studies, an intermediate room, and a workshop where the growing collections were housed and his work was done In 1871 he acquired, with his brother, the house Highcliff, at Lyme Regis at the far western end of the Dorset coast The fine diversified country with glorious coast scenery of this neighbourhood gave him constant and deep pleasure He laid out the garden afresh with marked success, and made alterations and additions to the house, again from his own plans Here some of the cooler months were spent each year with keen enjoyment by my father and his family Unless visitors were staying in the house and some larger expedition was planned, the morning, and often the early afternoon, would be spent by him in scientific work, but time was always allowed for some long country ramble with his children before dinner-time—or rather before dinner—for his enjoyment in the out-of-door life was so great that he was not a model of punctuality Until the later years of his life he would return to the drawing-room after the pipe which followed dinner, and read aloud himself or listen to reading. He was an excellent reader both of prose and poetry Novels he enjoyed if not too analytical of "poor human nature." Scott was a favourite, and Stevenson and some of Wilkie Collins' Kipling's 'Jungle Books' he greatly enjoyed, biography and books of travel were also welcome Later in life, however, he preferred to remain in the smoking-room, and then one of his daughters remained and read with him. Huxley's 'Essays,' works on Geology and Astronomy, and a good deal of other fairly stiff reading were gone through and well digested by them

The other months of the year were largely devoted to business and the public service. As he was able to share the burden of his business with his partners and had more leisure, he took up a variety of public work In his own religious body he served as clerk to the monthly meeting, administering the business of the Society, and taking a leading part on educational and philanthropic committees He never spoke in meeting (*i e* in meetings for worship) though he took his turn as reader in the Bible reading with which the Sunday morning session began He chose his seat at the end of a bench near a doorway looking straight out southward into the meeting-house garden (a but little modified piece of forest land) so that the contemplation of the outer world mingled with his inner reflections I think there must still be the marks and dates, cut after meeting, with his knife in the floorcloth (rather to the scandal of some members), showing the positions reached by the shadow of the top of the doorway at noon on mid-winter and mid-summer Sundays—the latter close to the door, the former, of course, far back in the room.

He was a very active member of the West Ham School Board, and it was at his initiative that the Truant School at Fyfield, near Ongar, was built

and equipped. This enterprise he carried through with all his strength and enthusiasm. He regarded it as of the highest importance to be able to deal with truant boys with rigorous strictness, but also to keep them uncontaminated by the criminal associations to which they were subject in industrial schools He took great pains in the selection of teachers and endeared himself to them by the sympathetic interest he took in their labours

He was an active and valued member of the local bench of magistrates, no sinecure in a district including a large East London element He was glad to work hard, often sitting in the courts three days a week during the summer and early autumn months, and his brother magistrates gladly acquiesced, in their turn, in his absence at other times of the year He also gave much time and attention to the work of the Essex County Council

Notwithstanding these varied activities he was able to carry on a good deal of scientific work even at Leytonstone Epping Forest and Wanstead Park in the immediate neighbourhood furnished fine hunting grounds On moving down to Lyme in middle or late autumn he looked forward to months of continuous and happy scientific labour undisturbed by public cares

His third daughter, Gulielma, as she grew up, became his especial companion and assistant in his scientific work, and all his natural history pursuits She easily acquired his bird lore, and became at least as skilled an observer as he Her training, at Bedford College, had given her a good grounding in systematic and structural botany, and her fine skill as a draughtswoman was an invaluable asset in their common labours

While working at moulds and other fungi my father had met with representatives of the Mycetozoa in their sporangial stage Now regarded as a group of Protozoa, they were at that time usually classified with fungi. Their remarkable life-history soon engaged his eager attention He watched the hatching of the spores into the flagellate active stage, the transition from flagellula to amœbula and the fusion of these to form the creeping plasmodia, the sclerotial stage of this and the final development of the plasmodium into sporangia. He became very skilful in dealing with these organisms, in the various phases of their life-history

While hunting one day, in the winter of 1876–7, in Epping Forest he came on a mass of the brilliant yellow plasmodium of *Badhamia utricularis*, which has the habit, almost unique in the group, of feeding not on dead vegetable matter but on certain living fungi. It was spread over a growth of the fungus *Stereum hirsutum* which had sprung from a hornbeam stump. The whole was brought home and carefully protected and observed Parts were allowed to pass into the dry sclerotial phase in which the protoplasm, having assumed the condition of a mass of minute cysts, is able to retain its vitality for months or even years, resuming its activity on being wetted and supplied with the proper food material A fragment of the sclerotium thus revived will grow rapidly if properly fed, so that in a few weeks a film of yolky protoplasm covering a soup-plate full of *Stereum* may be obtained from a morsel no bigger than a pin's head. This mass furnished the

material for renewed observation of its marvellous properties for several years

A meeting of the Linnean Society (February 15, 1877) at which my father exhibited some of the plasmodium of this gathering in active streaming condition (an object which very few had at that time ever had an opportunity of seeing) is well remembered by those who were present. The mysterious rhythmic backward and forward flow through the vein-like channels of the film of undifferentiated protoplasm is indeed a most striking phenomenon. His was no dry and lifeless exposition, he stood rather as one who had ascended into the Mount of Vision and whose high privilege and urgent duty it was to reveal what had been vouchsafed to his view. This was, in fact, his attitude of mind to all the phenomena of nature, whether the ways of beast or bird, the structure of plants, geological or physical phenomena, or the movements of heavenly bodies. It was all a revelation of the mystery of life or of the environment of living things on the earth and in the universe. When moved to speak of these things he would cast aside a shyness which had clung to him from his boyhood, and discourse with a force and eloquence which carried conviction to the hearers and enlisted their sympathies in the cause.

From this time onwards his attention was more and more concentrated on the Mycetozoa. The species were diligently collected on his rambles, compared with the published descriptions, and accurate and beautiful drawings were made of them. Many ladies, friends of the family, lent willing aid, and samples and small collections came dropping in. He soon started a large "ledger," in which references were entered to all notes and drawings of the various species scattered through his note-books, as well as to the specimens themselves, stored in small cardboard boxes, and to microscopic slides on which specimens were mounted in glycerine jelly. He published papers from time to time, at first in his own name but soon in association with his daughter, describing new species or interesting variants from types already described. He was, of course, familiar with the writings of de Bary, whose classical investigation of the Mycetozoa first demonstrated their position in the zoological rather than the botanical kingdom, and he regarded it as a high privilege when Prof. I. Bailey Balfour handed over to him for examination the collection which he had made years before at Strasburg when working under de Bary. Subsequently Greville's collection from the Herbarium of the University of Edinburgh was similarly lent him. He eagerly cultivated any germ of enthusiasm for the group, whether in his own circle or among correspondents, and he soon had a large number of friendly workers and collaborators, both at home and abroad. Every letter of interest was duly entered in the ledger, and he reckoned no pains wasted which were spent on his carefully considered (and duly copied) answers, which were generally accompanied by boxes of typical specimens. His daughter had set herself, some time before, to learn to read Polish, in order that the important systematic work of Rostafinsky, another of de Bary's pupils, might be at their disposal.

In 1892 he was invited by Mr Wm. Carruthers, the head of the Botanical Department of the British Museum, to prepare a Descriptive Catalogue of the collections of Mycetozoa in the Museum, and into the preparation of this he and his daughter threw all their energies Lodgings were taken at Kew while the collections in the Herbarium were overhauled, and a pleasant visit was made to Strasburg, where were de Bary's collections in the guardianship of his successor in the Chair of Botany, Graf zu Solms Laubach From him they had a most friendly reception, and it was a great pleasure to all the family when he paid a return visit, a few years later, to the home at Lyme

The catalogue, when complete, took the form of a monograph of the group It was published in 1894 My father also presented to the Museum samples, whole and mounted on slides, of all the species and varieties known to him, and the show case of the Mycetozoa in the Botanical Department is enriched with beautiful water-colour drawings by his daughter, giving magnified views of typical specimens of the group He took a patriotic pride in thus making the collection in the British Museum as complete as it lay in his power to make it.

The monograph had a sale unwonted in the series, and my father was invited to prepare for the Mycetozoa one of the small paper-covered guides to the collections which are issued by the departments, and this also attained considerable popularity

The publication of these works, far from bringing any pause in their labours, led, on the contrary, to a great increase in their circle of correspondents and in the specimens referred to them for examination

The United States, where the group has been much studied, the West Indies, New Zealand, Japan, Java, Borneo, Ceylon, and, in Europe, Germany, France, Scandinavia, Portugal, Switzerland—many were the post-marks on the small neat packages or large cases which frequently arrived from abroad

All this demanded long hours of strenuous labour from my father and his daughter, and it was often not till driven out by the growing dusk that they started on their accustomed ramble. Papers were published as before as material accumulated, dealing with new and interesting forms.

From the abundant material from all parts of the world which thus came to him, all carefully digested and illustrated in the note-books, my father and his daughter came to possess a knowledge of the typical characters of the species and the range of variation of which each is capable, which was unequalled. A second edition of the monograph was soon called for and it was to the preparation of this, strenuously carried on notwithstanding failing health and powers, that the later years of his life were devoted. It has been published since his death by his daughter (1911). The beautiful illustrations, many coloured, are far superior to those of the first edition (partly the result of the progress which has been made in late years in the art of mechanical reproduction), and the text embodies the mature results of their joint labours on the specific characters of the group Workers

at Mycetozoa, especially beginners, are apt to publish a description of some aberrant form, which they regard as a new species, but a larger survey will frequently reveal the aberration as one of many variants grouped about and merging into a type form, and quite unworthy of specific distinction It is the width of survey and the constant endeavour to approximate the classification to the relation and variation of the species of Mycetozoa as they occur in Nature, under varying conditions of climate and locality, that confer on the second edition of the monograph its unique authority

Although his chief endeavour was concentrated on this purpose, many of the advances in the knowledge of the life-history and physiology of the group were made by him or one or other of his children The first observation of the ingestion of living bacteria by the swarm spores (flagellulæ) was his, and also that of the peculiar mode of division of the spore contents of the Exosporeæ after their escape from the spore wall The remarkable simultaneous division by karyokinesis of the nuclei of the active plasmodium was first seen by his son, and Strasburger's observations of the karyokinetic division of nuclei prior to spore formation in *Trichia* were confirmed and extended to other genera and species. The complete life-history of the group, and especially the point at which gametic union occurs, are still undetermined

My father became a Fellow of the Linnean Society in 1873 and of the Royal Society in 1898. He was a member of the Essex and Dorset Field Clubs, and he was President of the British Mycological Society only two years before his death

He was very fond of foreign travel, and it was remarkable how, after he had been occupied for months with what might appear the work of a narrow specialist, often hardly talking of anything besides his beloved "Creepies," once free of his labour his mind responded to the charms of travel. He rejoiced in life on board ship, and never suffered from sea-sickness. He twice visited the North American continent, and his tours in Europe and Egypt with members of his family were often vividly recalled by him.

It was very rarely that he would allow himself to be enticed into paying visits in England, yet he had often immense enjoyment in them when once he had been induced to leave home.

"Labour and Sorrow" are, as we know, the frequent attendants of the declining years of a strong man's life, and to this his was no exception. He suffered more and more from bronchitic attacks, to which he had always been liable, and from other bodily ills Between the attacks, however, he often attained good measure of enjoyment in life, even in his later years, and owing to the loving devotion of his daughter, on whom, as his powers failed, he came to rely more and more, he was able to carry on his scientific work almost to the end As I have said, she has been able since his death to embody his riper knowledge of the Mycetozoa, and her own, in the second edition of the British Museum monograph All that loving care could do to smooth his path was done by his wife and the other members of his family.

He died rather suddenly at his home at Highcliff, Lyme Regis, on Sunday,

ALBERT GUNTHER

July 29, 1908, in his 79th year. He is buried in the burial ground of the Wanstead Meeting House, near Leytonstone, close to the oak tree on which he so often looked out as he sat in Meeting

The closing years of his life were clouded for himself, and those who were near him, by his failing health and powers It is to their recollections (if a son may be permitted so to write of his father) of the strong, wise, and loving man that he was in his prime, still attended by clear glimpses of the "Vision Splendid" and with much of the character, to use another Wordsworthian phrase, of "Nature's priest," that his family and his friends look back for the full evidences of the manner of man he was

J J L.

A C L G GÜNTHER, 1830–1914

Albert Günther was born in Esslingen, South Germany, on October 3, 1830, a descendant of a family which had been in the locality for hundreds of years, the Swabian branch of the Gunthers having settled in and about Möhringen on the Filder Plateau at the beginning of the fifteenth century. His father was "Siftungs-Commissar" in Esslingen, and Estates Bursar in Möhringen, whilst his mother, Eleonora Nagel, was a daughter of a family which originally came from Bremen but had been resident in Wurtemburg for four generations *

He obtained his early education at the Gymnasium at Stuttgart, and thereafter proceeded to the University of Tübingen, where he spent six years, 1847–52, 1856–7, the intervening years being occupied by attendance at the Universities of Berlin (1853) and Bonn (1854–5) This prolonged student-life was mainly due to the wishes of his relatives, who, according to family tradition, had destined him for the ministry of the Lutheran Church He attended, indeed, the Theological College at Tübingen, and passed the qualifying examination But the young student's bent lay in another direction, and, just as his brother turned to medicine, so he gravitated to natural science, especially after falling under the influence of the renowned Johannes Müller, who was then in the zenith of his fame, though a fatal accident which had happened to a student on one of his dredging expeditions had seriously affected him. He accordingly, after taking the degree of Ph.D. at Tübingen, in 1852, decided to study science and medicine, choosing zoology as the chief field of his labours, as evinced by his first paper, "Ueber den Puppenzustand eines Distoma," which appeared in the 'Württemberg

* 'Günther Family Records,' Quaritch, London (1910).

Jahreshefte' in 1853, in the same journal by his second note, "Beitrage zur Fauna Wurttembergs," by his "Fische des Neckars" in 1853, and by his 'Handbuch der Medicinischen Zoologie' in 1858 He fully qualified himself, however, for medicine, even studying for a time in St Bartholomew's Hospital, and graduated as M D at Tübingen in 1858. Thus he also illustrated the indissoluble brotherhood between medicine and zoology, of which Prof Allman (himself an M D) made so much in his introductory lecture in Edinburgh University in 1854, and which has been a striking feature from the earliest times till now.

The "Fische des Neckars"* gives an earnest of that methodical habit, accuracy, and patient investigation of his later years, both as regards the systematic examination of the conformation of each species, its size, colour, fins and fin-rays, scales, skeleton, eggs, parasites, and haunts It is a model of what a local fauna should be About thirty species, including the Cyclostomes, were entered, and at that date the young (so-called Ammocœte) was considered a separate genus This paper appeared also as a separate treatise with a finely coloured plate of *Leuciscus muticellus*, Bonap, which the author had detected in the river, and which was drawn by Prof Rapp †

The 'Handbuch der Medicinischen Zoologie' (1858) must have been a useful treatise for medical and other students It commences with the higher mammalia, concludes with the infusoria and sponges, and touches most forms of service or of interest to the student, and, without being too prolix, it gives a comprehensive grasp of the subject—specially alluding to the medical products derived from the various forms It does credit to the studious and earnest author even at this early period of his career

Visiting his mother in England in 1855, he met Dr John Edward Gray and Prof Owen in the British Museum, and both, having a knowledge of his previous work, took an interest in him, and a friendship sprang up between them Two years later Dr Günther was selected to arrange and describe the Fishes, Amphibians, and Reptiles in the National Collection, which task, sufficiently onerous then, became increasingly laborious as time advanced, whilst the work at first had little relation to adequate financial inducement. The eager naturalist cared little for the latter if only the opportunity for extending knowledge, and for placing the varied collections committed to his care on a proper footing, were given him, and from October, 1857,‡ onward, he devoted himself to this task. Those familiar with his cellar-like apartments in the old Museum will appreciate the enthusiasm and unswerving loyalty, as well as remarkable ability, he brought to bear on his work, apparently indifferent to depressing surroundings and formidable difficulties connected with literature and specimens.

In glancing at the remarkable list of memoirs (246) and papers entered

* 'Württembergische Naturwissenschaftliche Jahreshefte,' Stuttgart, 1853.

† For much information contained in this notice of Dr. Günther I am indebted to his son, Mr R. Günther, M.A., Fellow of Magdalen College, Oxford.

‡ They started him with 2,000 bottles of snakes in 1857.

in the Catalogue of the Royal Society the reader is struck by the variety as well as by the laborious nature of most of them. Few men have produced such a series of meritorious contributions to our knowledge of zoology, ranging from Distomes and Spiders to Mammalia, but chiefly concentrated on Fishes, Amphibians, and Reptiles, though Birds and Mammals come in for a considerable share, and yet this list is incomplete—without for the moment alluding to his separate works Some of his earlier papers appeared in the 'Wurttemberg Jahreshefte' and in 'Wiegmann's Archiv,' but after he was settled in the British Museum the majority of his valuable contributions were published in the 'Proceedings' and 'Transactions' of the Zoological Society, those of the Royal Society, and in the 'Annals of Natural History'—of which he was so long the chief editor His lucid and terse descriptions of new fishes, frogs, snakes, lizards, tortoises, of birds and mammals, his anatomical memoirs on *Hatteria* (*Sphenodon*) and *Ceratodus*, his descriptions of zoological gardens at home and abroad, his papers on the distribution of reptiles, on zoological nomenclature, and on fossil fishes were sufficient for the foundation of several reputations Every collection of note made by explorers all over the world came to him at least for the fishes, frogs, and reptiles, and occasionally for the birds and mammals Special memoirs on certain groups were intermingled with faunistic reports and descriptions of new forms in the British Museum, ranging from an undescribed spider from Cochin China, a new species of long-tailed titmouse, the insectivorous mammal *Potamogale*, to a new poison-organ in batrachoid fishes, and the skeleton of *Ausonia* Further, as the founder and first editor of the 'Zoological Record' in 1865, he placed naturalists under a debt of gratitude which continues now with unabated force, since the modern developments in this field are largely due to his original efforts His object, "to acquaint zoologists with the progress of every branch of their science in all parts of the globe, and to form a repertory which will retain its value for the student of future years," has been amply borne out.

His incisive criticism (1859) of the work of a Continental author on the snakes is incorporated, again, in his description of a new genus of West African snakes (*Elapops*), and revision of the South American *Elaps*, whilst his researches make a distinct advance on our knowledge His historical account of *Echeneis* (1860) reveals not only the classical knowledge of the author, but an intimate acquaintance with the extensive literature and of the rich collections in the British Museum, so that he was enabled not only to correct previous errors, but to add two new species to the genus.

Most instructive was his paper (1859) on the sexual differences in recent and fossil frogs and fishes, and especially of *Ceratophrys*, the female having a skull about three times as large as that of the male Its peculiar and solid structure in both sexes is due to the mode of life of these frogs, which feed on other frogs, birds, mice, and young rats, thus Dr Günther found in the stomach of one a *Cystognathus* half the size of its destroyer, for, with its wide cleft and enormous cavity of the mouth, its powerful muscles from the

tympanic and neighbouring bones, and short teeth, it securely holds its prey. These parts, indeed, form a contrast with those in other Anura. His acuteness in discriminating the fossil humerus of the male *Cystognathus*, which is specially developed in connection with propagation, rested on his thorough knowledge of the living forms, and so with the presence of the thick ray in the pelvic fin of the Tench

He notes that the inhabitants of the Sandwich Islands search the tidal pools at low water for small fish-fry, and convey them to ponds (fresh water) "in which in a short time they increase to a size fit for the table" (E T Bennett, formerly Secretary Zoological Society) Dr. Günther adds (1861) that though he considers the acclimatisation of foreign fishes as a matter of subordinate value from a practical point of view, it is a problem of high scientific importance, because it involves the solution of the question, how far the power of man is able to interfere with the original distribution of fishes He advises the selection of forms from a similar climate, *e g* the Wels (*Silurus glanis*) of the Continent If tropical forms are wished, the Gorami (*Osphromenus olfax*) a freshwater fish reaching 15 lb, and which has been introduced into Mauritius and Cayenne, the climbing Perch (*Anabas scandens*), and the Pla Kat of Siam (*Betta pugnax*) as deserving trial.

The grasp which, even at this early age, Dr Gunther had obtained of the distribution of Reptiles and Batrachians is evident in the masterly paper communicated to the Zoological Society in 1858. A careful perusal of this demonstrates that the author had forestalled many interesting features which have since been described by others. The contrast between snakes and amphibians in connection with temperature and temporary physical disadvantages is pronounced, and this makes snakes well adapted for clearing up the question, Creation *versus* Evolution. He contrasts the reptilian distribution with that of birds (which P. L Sclater had communicated to the same society a few months earlier, and which had often been the subject of discussion between the two naturalists), and it is possible that the views of Dr. Günther had some influence even in regard to the birds. He united, however, the Ethiopian shores of the Mediterranean with the Palæarctic region, instead of considering Spain and Portugal as approximating more to Africa than to Europe, as Schlegel did He differed from the latter also in showing that a snake like *Dasypeltis scaber*, living on trees in Africa, devouring eggs of birds, the shells of which it breaks by gular teeth and with irregular arrangement of the lateral scales, cannot be a representative of the genus *Tropidonotus* The Hydridæ of the Indian region, the linking of Japan to this region, the large proportion of venomous snakes in Australia, the two systems radiating from the Mississippi in the north and the Amazon in the south in the Nearctic region, the comparative paucity of snakes in the Neotropical region, are all forcibly portrayed in this communication. In the same way the peculiarities of the distribution of the Amphibians are dealt with throughout the several regions, many striking facts being brought forward for the first time. The total absence of Batrachians from New

Zealand, the absence of the tailed forms from Tropical Africa, the Arctic character of the Batrachian fauna of Japan, whereas its snakes are Tropical, the absence of *Hyla* in India and Africa, the spread of the European *Rana temporaria* to the Nearctic region and its absence in the Neotropical, and the resemblance of the Batrachian fauna of South America to that of Australia, are a few of the salient points of this important communication In after years he was enabled to supply Mr Darwin with so many interesting facts relative to the reproduction and the wedding-dress of fishes, amphibians and reptiles, and to such an extent that Darwin wrote "My essay, as far as fishes, batrachians, and reptiles are concerned, will be in fact yours, only written by me"

To Alfred Russel Wallace's 'Geographical Distribution of Animals' Dr Günther contributed much important information concerning the distribution and classification of fishes and reptiles, the gigantic tortoises of Galapagos and the Mascarene Islands, the height to which reptiles reach on the Himalayas, and on the distribution of fishes—especially the identity or close affinity of those occurring on each side of the Isthmus of Panama, rendering it probable that Central America has been partially submerged up to comparatively recent geological times His researches on the freshwater fishes of the same region would point to a like conclusion, seeing that a number of fish-faunas can be distinguished, corresponding to some extent with the islands into which the country would be divided by a subsidence of about 2000 feet, the most important of the divisions separating Honduras from Costa Rica.

His work on the Reptiles of British India, published by the Ray Society in 1864, is a systematic treatise of great merit, for not only does the author give the results of his labours in the British Museum, but he examined every available collection at home, and included those of Burma, Siam, Cochin China, and Southern China The philosophical spirit in which he dealt with the genera and larger groups is manifest throughout, and the 26 lithographic plates, chiefly by Ford,* can hardly be surpassed The work is a monument of patient labour, wide knowledge, and scrupulous care

His careful account of the anatomy of *Hatteria* (*Sphenodon*), in 1867, enabled him to make a step in advance in the classification of recent Reptilia, a step which zoologists have since followed in connection with the characters of the Rhynchocephalia The fixed quadrate, cartilaginous ali- and orbitosphenoids, the union of the mandibular rami by ligament, the uncinate processes of the ribs, double temporal bars, amphicœlous vertebræ and absence of copulatory organs, showed characters of ordinal importance Researches since that period have borne out the prediction of the author that discoveries of extinct allied forms would further add to our knowledge Thus the group of the Prosauri with its sub-order Proterosauri including *Palæohatteria* from the Lower Red Sandstone of Saxony, and

* He greatly pleased Mr Darwin by getting this skilful artist, in 1870, to do his wood-engravings.

Proterosaurus from the upper Permian of Thuringia and Durham, show how correctly Dr Günther had anticipated the extension of the group.

The treatise, along with Lieut.-Colonel Sir Lambert Playfair, on the Fishes of Zanzibar, added many new species to the fauna of the East Coast of Africa, and by the patronage of the Government of Bombay the authors were able to illustrate their volume with finely coloured lithographic plates by Ford.

The eight volumes of the Catalogue of Fishes in the British Museum is a work of extraordinary research, the list of the authors and their works alone occupying, for example, in the first volume of the Acanthopterygian Fishes, about 12 pages Thus in the first two volumes the number of species in each is nearly double that of Cuvier and Valenciennes, the last general ichthyological work, more than double in the third, and so on throughout the series The labour of examining the descriptions and determining species by them, of correcting erroneous interpretations, and of giving an account of each which would have the "distinctness of a diagnosis and the accuracy of a description," must have been enormous, not to allude to the task of going over the numerous special and ever increasing collections in and beyond the Museum, and correcting the synonymy. It is not generally known that the author worked at the first three volumes under great disadvantages, especially in regard to financial aid and time, facts which should be borne in mind in their review The vast area from which the collections were drawn sufficiently explains the nature of the undertaking, since Arctic and Antarctic, Temperate and Tropical seas and fresh waters, were equally ransacked for their fishes Yet in the second volume he was far from being satisfied as to the completeness of the task, since so many forms entirely new to science had rewarded him, that he urged the collection of fishes in every country, for, he added, "we may well conclude that not one-tenth of existing species are known" (1860)

In his progress with the task allotted to him, he states that though weighty reasons have been brought forward against the natural limits of the Acanthopterygian order of Johannes Müller, he still feels satisfied with Muller's ordinal arrangement, and is of opinion that no character is of equal importance to that of the structure and position of the fins, and that the number of the vertebræ is of great value in distinguishing families He, however, shared the opinion of those who consider the coalescent pharyngeal bones as of sufficient importance to unite acanthopterous and malacopterous fishes into one order, and changed the name *Pharyngognathi acanthopteri* into *Acanthopterygii pharyngognathi*. His *Acanthini* coincide essentially with the *Malacopterygii jugulares* of the old authors, and he observes they are a very natural order His experienced remarks concerning the cod and the sole are worthy of remembrance, some ichthyologists placing them indeed in distinct orders, but the absence of symmetry is the only constant character on which such an opinion can be founded, and it is but little developed in the more highly organised forms such as *Psettodes*, a genus in which the asymmetry is almost entirely limited to the position of the eyes,

which are on the right side in one half of the specimens and on the left in the other. The vast literature in such a family as the Salmonidæ absorbed as much patience and time in the investigation of a single species as in other fishes for that of a whole family. The small family of the Umbridæ, however, gave Dr. Günther some compensation, since of its two species one occurred in Central Europe, the other in North America, the close affinity of the two being recognised by him for the first time, and constituting "one of the most striking instances against the geographical continuity of identical forms." In the Preface to his eighth volume many valuable observations were made, some of which are widely applicable. Thus, in defining a species, he considered it to be well established only when it is founded on characters which, from an examination of numerous examples, are found to be permanent, not subject to gradual variation, and not dependent on season, sex, or age, or which are known to be so from the examination of allied forms. An idea of the extensive and laborious nature of Dr. Gunther's task is gained by his statement that 6843 species of fishes in his Catalogue are well established and described, whilst 1682 others are doubtful. "Assuming that about one-half of the latter will ultimately be admitted, and that since the publication of this work 1000 species have been described elsewhere, *we may put the total number of fishes known at present as about* 9000." At the date of publication of the last volume, he calculated there were 29,275 examples of fishes in the British Museum, a vast array, largely due to his own initiative, and to his personal influence, yet he modestly states that it contains not two-thirds of the known species, and instances the gaps yet present, even in such well known forms as the herrings, the sharks, and the rays. He therefore urged the necessity of keeping pace with the rapid progress in ichthyology resulting from the efforts in other countries, adding that no other class of vertebrates offers a similar gradation of development of the most important systems and organs, rendering its systematic arrangement a most difficult problem. His further remarks as to the importance of fishes in elucidating the geographical distribution of animals and the relations of the various epochs to one another doubtless proved an invaluable aid in determining the importance of such an Expedition as that of the "Challenger." He modestly concludes this epoch-making labour by stating that, "if it should assist my fellow-labourers and enlist others—if it should contribute to the advancement of truth, I shall not repent having devoted the best years of my life to its execution."

Early in his career, Dr Günther kept in touch with the Museum Godeffroy at Hamburg; indeed, in 1868, he was instrumental in enriching some private collections of invertebrates through this agency, for the staff of the ships employed by the firm of merchants specially collected every group. Dr. Günther undertook the Monograph on the South Sea Fishes, thereby making a noteworthy addition to his famous works both in text and illustration, since the artist, Andrew Garrett, had lived in the Pacific Islands and made drawings from life of all the fishes which fell in his way, just as the late Colonel Drummond-Hay, of Seggieden, did with those off the Bermudas,

but with this difference, that Garrett's specimens and drawings went to the Museum Godeffroy, whilst Colonel Drummond-Hay's specimens were lost by the thirst for alcohol of a man left in charge during the absence of the Colonel in Britain Portions of Dr Gunther's work, which was undertaken on condition that a selection of the fishes, including all the types, should be presented to the British Museum, were issued in 1873 and 1881 A long blank, mainly due to financial reasons, then occurred, and it was fully a quarter of a century later before Friedrichsen, the publisher, wrote to Dr Günther asking if he could proceed, since Dr Martin Godeffroy had now advanced funds Thus, after his retirement from office, the veteran ichthyologist was enabled to complete his great task in 1909 and 1911 This fine work could only have been accomplished by one with an encyclopædic knowledge of fishes and their synonymy, and in touch with the vast collections in the British Museum, and the gorgeous coloration of many of the fishes and their interesting habits make the work of special interest and value

Having to re-write the article on "Ichthyology" in the Eighth Edition of the 'Encyclopædia Britannica' originally prepared by Sir John Richardson, Dr Gunther took the opportunity of publishing an 'Introduction to the Study of Fishes' (1880), a treatise which, in limited compass, places before the student the history and literature of the subject, the structure, growth, and variation of fishes, their distribution and systematic relations. This work is invaluable to the student and ichthyologist, and is especially interesting in those chapters dealing with the distribution of fishes, and the problems opened up by the appearance of identical families, genera, and species in distant continents, such as, for instance, *Galaxias*, in Southern Australia, New Zealand, and the southern parts of South America A German translation of this work was published in 1886

When elected to preside over the Biological Section of the British Association in those palmy days (1880) when zoologists, botanists, physiologists, anthropologists, and the rest all fell under this head, he chose as the subject of his address that which his predecessor, Dr J. E. Gray, had chosen before him (1864 ?), viz, "Museums their Use and Improvement," and his experienced remarks were worthy of the theme He made three groups of museums—(1) National, (2) Provincial, and (3) Educational—though these pass into each other and there may be hybrids between them He gave an outline of the new Natural History Museum at South Kensington, pointing out that in this, the greatest National Museum, it would be impracticable to group the recent with the fossil forms, however strongly the principle of studying the two may be held, for all agree that zoologists and botanists should not be content with the study of the recent fauna and flora, nor should palæontologists carry on their researches without due reference to the living forms. In the museum two series are necessary, viz., those illustrating the leading points of popular and scientific interest, and, secondly, the study-series. He also emphasised the construction of cases of metal as a substitute

for wood, and in this he anticipated what thirty years later became the rule in the finest collections In insisting on the formation of a Natural History Library in connection with the National Collection, and in the policy of distributing duplicates to provincial museums, he held enlightened views, which have since been fully acted on

His work on the Shore Fishes of the "Challenger" (1879) showed that even the limited opportunities of the naturalists for making such collections were productive, for the series consisted of 1400 specimens, of which 94 were new to science Yet the chief efforts of the explorers were devoted only to such localities as were previously more or less uninvestigated, and which were rarely visited His familiarity with the subject and his methodical method of working enabled him to issue this volume whilst Sir Wyville Thomson was still at the head of the "Challenger" office Dr Gunther took the opportunity of widening our knowledge with regard to the mutual relations of the fishes of the deep and shallow waters, and of demonstrating the wide range of many, both as regards depth and locality He fixed the dividing line between these and the deep-sea fishes at 100 fathoms, though Sir Wyville Thomson and he at first thought that the dividing line should be from 300 to 350 fathoms, and his grounds were that "no fish not known at present to have occurred beyond the 100 fathom line is admitted in the Report, and, further, that no truly bathybial fish is known to live habitually above that line"

In his great work on the Deep-sea Fishes of the "Challenger" (1887) he combined all the information gained during the subsequent productive cruises of the "Knight Errant" and "Triton," as well as such new materials as could be gleaned from the fragmentary and preliminary notices of the expeditions by the two Institutions of the United States, and by the expeditions of the French, Norwegian, and Italian Governments The total specimens were referred to 266 species, of which 177 fell to the share of the "Challenger" and 14 to the exploration of the Faroe Channel The number of new forms amounted to no less than 144, whilst 10 were added to the fauna of the British seas. Every trained zoologist will coincide with his concluding words in the preface, viz "My technical descriptions of the Challenger fishes will be found to be much more concise than those given by some recent writers . . . . the practice of circumstantially describing every minute detail of the surface of a fish, repeating every point of structure common to all the species of the genus or family, and indiscriminately mixing individual characters with specific, not only renders the use of these lengthy descriptions a laborious and thankless task, but actually leads to misunderstandings not less frequently than the insufficient short diagnoses that have been prepared by inexperienced describers."

In this valuable treatise he first gives a careful historical digest of the subject, referring especially to the work of the Norwegian North Atlantic Expedition and to that of the United States Fish Commission under Prof. Alex. Agassiz and Dr Spencer Baird, the former expedition reaching

1400 fathoms and the latter 2900 fathoms, a similar depth having produced fishes in the "Challenger" No part of this fine treatise is more interesting than that in which the characteristics of the deep-sea fishes are portrayed by the author, such as the size of the eyes, the black colour of the pharynx and of the surface of the cœlom, the fibrous, fissured, and cavernous structure of the feebly developed bones, the thin lateral muscles, and the loose connection of the vertebræ. As a consequence, when they are drawn to the surface these specimens require the most careful manipulation to prevent their breaking into fragments Yet under the normal conditions of their abyssal home, that is under the enormous pressure of the surrounding element, the fibro-osseous tissues and the thin muscles suffice for rapid and powerful movements. When drawn up the expansion of the gases in the air-bladder causes the gullet and stomach to be thrust out of the mouth and the eyes from their sockets. These deep-sea fishes possess a largely developed muciferous system on head and body, and in addition a series of phosphorescent organs Dr. Günther gives a lucid and comprehensive description of the modifications of these organs as to distribution, appearance and structure, grouping his remarks under nine heads He considered that these fishes contribute to a considerable extent to the luminosity of the abyssal depths, and that such light enables the possessor to see, and in those in which the organs are highly developed and specialised, the light is under the will of the fish, which thus can use its "searchlight" for the purpose of discovering prey or for other purposes. Further, the occurrence of such organs in the cavities of the gills or within the mouth does not invalidate such a view, as the membranes and bones are semitransparent. On the other hand, he pointed out that the luminous organs which are placed on barbels, filamentous fin-rays, or tentacles serve as lures, and that even those on the caudal peduncles of Scopelids, Sternoptychids, and others probably have the same function

Another notable observation of Dr Günther's was the reduction of the gill laminæ in these fishes: the horny rods which support the plaits of the mucous membrane being deficient in firmness, the laminæ are reduced in number and the respiratory surface diminished He was inclined to associate this with their sojourn in the low temperature of the abyssal depths and its effect on circulation and respiration. He also points out that the spawn of some of them (*e g Polyprion cernuum*) develops at the surface, whilst the young fishes, after a short pelagic existence, descend to the bottom as in the flat fishes. He considers, however, that in others the spawn will be deposited on the bottom and hatched there, thus affording an extreme contrast to the former, which were developed under the accelerating influences of light, warmth, and a constant supply of oxygen. The oviposition and hatching of the eggs of fishes, indeed, are marvellous in their infinite variation.

Equally interesting are his remarks on the vertical and horizontal distribution of these deep-sea fishes, the families, for instance, which descend to the greatest depth, viz. 2900 fathoms, are Berycidæ, Pediculati, Ophidiidæ,

Macruridæ, Sternoptychidæ, Scopelidæ, Stomiatidæ, and Murænidæ, besides the Alepocephalidæ, and the Halosauridæ, which have no representatives in the surface-fauna He shows that the abundance and variety of fish-life decreases as the depth increases, and that the uniformity of the physical characters of the sea-bottom gives rise to the almost unlimited horizontal distribution of deep sea fishes, so that the same genera and even the same species may occur in the depths of the eastern, western, northern, and southern hemispheres.

The whole of this fine work teems with novelties in the structure of the remarkable forms so carefully dealt with by the author, and which were graphically illustrated by the brothers Mintern

One of the most interesting labours was his final one on the History of the Zoological Department of the British Museum from the year 1856 to the year 1895, when Günther retired from active service, and which was published by the Trustees at the end of 1912 This record of 39 years' experience of the National Collection is told with great accuracy and rare modesty, and perhaps more than any other evidence testifies to the zeal, perseverance, and popularity of the Keeper—even under circumstances not always conducive to progress. The increased grant, from £1100 to £1500 per annum, during this period enabled the Keeper to effect greater uniformity in the growth of the branches of the collections, and to follow Dr Gray's plan of forming a study-series as well as an exhibition-series The collections in 1856 were well arranged in cases, and the specimens were clean and well preserved, whilst the richness in rare types made the Museum even then not behind those on the Continent Those who knew the collections at the former date, however, can appreciate the vast changes which were inaugurated in such departments as the mammals, birds, reptiles, and fishes, as well as in the invertebrates in general—especially during the period of Dr Gunther's keepership. Whilst he laboured at the specimens and catalogues himself, he also encouraged others in the same field Thus the catalogues on almost every group of note made considerable progress

The labours during his keepership (1875–95) may be dealt with under the following heads :—

1 *The Increase and Arrangement of the Collections*—The appointment of Dr. Günther as Keeper in 1875 was followed by a great increase in the collections generally, by such additions as those of the "Transit of Venus" Expeditions, the Arctic Expedition, collections by naval officers, the "Challenger" collections, the Bowerbank collection of Sponges, the Gould collection of Birds, the Godman and Salvin collections of Birds, the Hewitson collection of Exotic Butterflies, the collections from the East India Company's Museum, collections from the International Fisheries' Exhibition of 1883, Zeller's Microlepidoptera, the Hume collection of Birds, Godman and Salvin's American Birds, the Tweeddale collection of Birds and Works, the Walsingham collection of Lepidoptera, the Godman North American Birds, the Day collection of Indian and other Fishes, the Keyserling Arachnids, the

Frey Lepidoptera, the Carter Sponges, the Parker Foraminifera, the Hume Heads and Horns of Large Game, the Anderson Egyptian Mammals, the Pascoe Coleoptera, the Stainton Lepidoptera, the Lilford Birds of Europe, F. Moore's Indian Moths, the Godman and Salvin Insects, Saville Kent Corals, and many others. An idea of the vast increase during Dr. Günther's period of office may be gained by comparing the census of 1868, viz, 1,000,000, with that in 1880, viz, 1,300,000, and in 1895, 2,245,000 Much of this increase was due to the constant efforts of the Keeper and his friendship with the leaders of expeditions, naval and military officers, as well as with naturalists at home and abroad.

In the arrangement of the collections increased progress was by-and-by obtained by the employment of temporary workers distinguished for their knowledge in certain departments, the Treasury being less reluctant to the temporary employment of such specialists than to additions to the permanent staff Thus Messrs. Seebohm, P L Sclater, O Salvin, E. Hargitt, and Count Salvadori aided in cataloguing the birds, Mr (afterwards Sir) George Hampson and Mr Warren worked amongst the insects, whilst Mr George Brook took (alas, only for a short time) the Madreporarian corals in hand. Others who aided in this work were Prof Rupert Jones in the Foraminifera and Mr H M. Bernard, who, on the death of Mr G Brook, took up his task Noteworthy advances were thus made by the combined labours of these skilled naturalists and by those of the staff.

Dr Gunther also instituted in 1875 a fascinating method of exhibiting the birds which breed in Britain, with their nests, eggs, and young exactly as in their native surroundings, only the perishable parts of the plants being artificially reproduced, whilst the actual parents and the makers of the nests were in every case secured No more popular part of the great Museum exists than the long Bird Gallery, in which these beautiful and interesting groups are exhibited, for the life-like attitudes and the suggestive surroundings appeal to the average citizen as well as to the cultured man of science. Dr Günther's experience as a field naturalist and aviculturist enabled him to grasp all the essential features in such a display, and to combine accuracy with the most charming effects. In his record of the collections he pays a tribute to Lord Walsingham's help in securing suitable specimens

2 *The Staff.*—When Dr. Günther received the appointment of Keeper, Mr F Smith succeeded him as Assistant Keeper in charge of the Entomological collections, and, in 1878, there were nine Junior Assistants, viz., Mr A G. Butler (Myriopoda, Arachnida, and Lepidoptera), Mr. C. O. Waterhouse (Coleoptera), Mr E. A. Smith (Mollusca), Mr E J Miers (Crustacea), Mr R Bowdler Sharpe (Birds), Mr. Oldfield Thomas (Mammalia), Mr. S. O. Ridley (Polyzoa, Hydrozoa, and Anthozoa), Mr. F. J. Bell (Worms and Echinoderms), and Mr O'Shaughnessy (Reptiles and Fishes). This staff Dr. Günther found to be insufficient for overtaking the labours entailed by the ever increasing collections, and the Treasury, in 1882–83, sanctioned a First-Class Assistant, Mr. G. A. Boulenger (Reptiles and Fishes), and two

Second-Class Assistants, Messrs. W. R. Ogilvie-Grant (Birds), and Mr J. J. Quelch (Polyzoa, Anthozoa, and Hydrozoa) In addition, an articulator and two boy attendants were appointed. A most important change was at the same time instituted by the re-arrangement of the duties of the attendants (13), many of whom were skilled manipulators, so that when they were relieved of menial duties they became of much assistance to the scientific staff in manipulating specimens, and in writing and copying. Some of these assistants had considerable knowledge of the collections, and were able, for instance, so early as 1863, to name collections for outsiders By this change the preparation of the various catalogues was expedited

3 *Catalogues and Guides*.—One of the duties of the Keeper was to superintend the preparation of catalogues and guides of the vast collections, and Dr. Günther from the first set himself with energy to this department, showing an example by his own Catalogue of the Gigantic Land-Tortoises, living and extinct, 96 pages and 55 plates (1877) A continuous series of catalogues and guides marked his tenure of office, and rendered the Museum a centre of zoological progress, as well as a popular resort for information in Natural History When the Trustees consulted the Keepers about the Index Museum proposed by Sir Richard Owen, the Superintendent, Dr. Günther's recommendation to them was as follows.—"To render the exhibition-series in every way instructive, a more perfect plan of labelling throughout the collection should be introduced, and a new guide-book should be prepared A clearly written guide, well illustrated with woodcuts, will supply all the information useful to the public and draw their attention to the more remarkable types. As the different divisions of the animal kingdom will be separated in distinct rooms, it will be possible to prepare this guide on an entirely different plan from that at present in use at the British Museum, viz, in the form of a popular, but systematic, handbook of Natural History" The Index Museum was proceeded with, though greatly altered in aim and constitution in subsequent years, and Gunther's suggestion in regard to the guide-books was successfully carried out, so that these were instructive not only to the visitors to the Museum, but to Provincial Museums and schools of Natural History all over the country

The plans of the new Natural History Museum at South Kensington were submitted to Dr. Gray in 1871, and when Dr Günther became Keeper in 1875, he and the other Keepers watched the arrangements for the several galleries as instructed by the Trustees. Yet he was greatly handicapped by the arrangements made by the architect, so that it was found impossible to have the mammals on the ground floor as intended, and thus the birds had to be placed there. It was during his tenure of the office of Keeper that the transfer of the vast zoological collections from Bloomsbury to their new home in South Kensington took place, viz., in 1882–83. The Superintendent, Prof. Owen, was then advanced in life, so that the chief responsibility fell on Günther, and the successful manner in which this delicate task was carried out reflects credit equally on his administrative capacity and his

ingenuity, and, indeed, the Trustees paid a tribute to Dr. Günther and his staff for "the successful removal of the zoological collections without any accident of importance," and acknowledging "their sense of the forethought and care shown in the direction of the removal, and of the zealous assistance of officers and attendants in effecting it." The enormous labour involved in the re-arrangement, for which careful plans of the galleries had previously been prepared, so that in many cases the specimens were placed at once in position, may be estimated by the fact that besides the removal of about a fourth of the collections in 1882, no less than 350 journeys were made by vans in 1883, in addition to the transfer of very delicate specimens in cabs or by hand. To the persevering efforts of Dr. Günther perhaps, more than any other, the design of a special Spirit Building is due, and it has the form of a large quadrangular hall with a floor and a roof of cement, besides other ingenious arrangements for the control of free spirit, and for the effective application of water in case of fire.

The formation of a Zoological Library had early been considered both by Dr. Gray and Dr. Günther, and the subject became more urgent when the transfer from the neighbourhood of the great National Library in Bloomsbury was decided on, yet it was not till 1879–81 that a commencement was made. Dr. Günther prepared the first catalogue of books in the Zoological Department, with the help of John Saunders, comprising 1700 titles, including 182 works. The Treasury allowed the unexpended balance (£5700) of the previous year to be devoted to this purpose, making a further grant of £5000 for each of the five following years. Dr Günther had taken upon himself the work connected both with the Zoological and the General Library in the new Museum, selecting, indeed, the books himself at Quaritch's, but, as the works increased in number, the Trustees granted for several years the assistance of Mr J. E. Harting in the Zoological Library. By purchase, presentation, or exchange the Departmental Library had, in 1895, amassed 10,036 separate works or 16,238 volumes, a sufficient proof of the constant care and thoughtful supervision of the Keeper, at whose suggestion John Saunders, who had been specially trained for this work, was placed in attendance on the Library. Thus one of the most important adjuncts to the National Collection was established, and has been of signal service to every member of the staff as well as to the numerous British and foreign workers who resort to the great collection for study.

In connection with the Library Dr. Günther arranged for the transference from Bloomsbury of several valuable collections of original drawings of animals, such as J. Abbot's original drawings of the Insects of Georgia—in 17 volumes, and of Major-General Hardwicke's drawings of Indian animals—in 33 volumes. This department is an important one and merited the attention the Keeper bestowed on it. He also formed a large private collection of zoological drawings, the arrangement of which formed the recreation of his leisure hours.

Dr. Günther's profound knowledge of fishes was utilised in public

inquiries, as, for instance, in distinguishing salmonoids, and was of much service to the public authorities in such cases, nor were his labours on the effects of pollution of salmon- and trout-rivers less important He carried out, for instance, careful experiments on the effects of the pollution of the lower Thames, noticing the length of time that fishes would survive in water tainted with sewage, the effluents from gas-works, and other injurious mixtures He, indeed, carefully surveyed the river in a steam-vessel placed at his service by the Metropolitan Board of Works In the case of the "yellow fins" of the Allan Water, again, his experienced advice was decisive, though a skilful lawyer, by presenting a Lochleven trout to another less wary scientific witness, created a diversion in favour of a contrary view

Throughout his life in England his fondness for pets of diverse kinds continually asserted itself. Thus in his young married life at Surbiton an artificial tree by the fireplace of his dining-room harboured a chameleon and a small parroquet, the former invariably and successfully contesting for the most comfortable perch with the latter—until on a dusky winter morning it left its bough to crawl on the carpet, the colour of which it assumed, and was unwittingly trodden on by a servant Tree-frogs uttered their curious notes from a Wardian case with its plants, and other species hopped about on the green moss beneath At Surbiton, also, he had a pet alligator, which he kept in his bedroom, and a giant tortoise in the garden along with the old-world *Hatteria* (*Sphenodon*) from New Zealand His aviary contained blue tits, cormorants (which were fed on fish offal and rats), a raven, hoopoes, and shrikes, whilst others occupied cages in the house A nest of young kestrels (now in the University Museum, St Andrews) shows how successful he was in the rearing of his pet animals, and the same may be said of his efforts with the Tussah silk moth of India. Many will remember his success in rearing for the first time the red-backed shrike in his aviary at Kew Gardens, for, though the first brood did not live to maturity, the second brood of five reached the adult condition, the parents feeding them especially on earthworms, which they cut in small pieces Moreover, he noted that their song imitated that of the garden warbler He took much interest in the nesting of a pair of storks in Kew Gardens, where several pairs still remain—a source of interest to ornithologists and the public, and an additional charm to the magnificent grounds. His remarkable grackle (a gift from Lord Lilford) at Kew Gardens was known to all his visitors, and its performances were a source of never-ending interest and amusement A small pond in his garden at Hampton Wick again gave an opportunity for observing the habits of fishes. When detained indoors by rainy weather, his active mind found exercise and recreation indoors in the care of his private collections.

Though devoted to his scientific labours, Günther was a delightful companion, and one of the kindest parents—ever ready to sacrifice himself for the happiness of his family, whose interests and welfare were to him paramount. As a host he will be remembered by many a man of science at home and abroad; and as a genial friend whose vast stores of information

were ever at the disposal of others. Delighting in the study of nature, he was equally at home in the country and in the metropolis, and many a useful hint he gave to those who in their rural retreats had devoted themselves to the fauna and flora around them. He was, moreover, an expert angler and a skilful shot

Dr Günther was in correspondence with naturalists all over the world, and was a member of many learned societies at home and abroad, was President of the Biological Section of the British Association, 1880, President of the Linnean Society, 1904, and Vice-President of the Royal Society He received the Royal Medal of the Royal Society, the Gold Medal of the Linnean Society, and the Medal of the Avicultural Society

As a systematic zoologist Dr Günther held a foremost position—whether we view the vast extent of his labours, their accuracy, or their importance. For nigh sixty years he pursued his studies with rare singleness of purpose, great natural ability, and conspicuous success—unmindful of those external encouragements which by some are held in great estimation. It was sufficient for him that he was advancing knowledge and doing his duty to the public, whilst the work itself was both spontaneous and pleasurable. His administrative labours in the British Museum alone and his skill in the transference of the great collections to South Kensington are remarkable and demonstrate the all-round nature of his accomplishments. He devoted his untiring energies throughout a long life to the advancement of science in its strictest sense

W C. M.

WALTER HOLBROOK GASKELL, 1847–1914

Walter Holbrook Gaskell was born on November 1, 1847, at Naples, where his parents were passing the winter for the sake of his father's health. His father, John Dakin Gaskell, was a barrister—a member of the Middle Temple—who followed his profession for a few years and then retired to private life His mother was Anne Gaskell, second cousin of his father Gaskell as a boy lived with his father at Highgate, and attended Sir Roger Cholmeley's School at that place. At school he worked chiefly at mathematics, but had considerable interest in natural history, and appears to have made more than the usual schoolboy collections connected with that subject

He came up to Cambridge in October, 1865, when he was not quite 18, as a member of Trinity College In his third year he was elected to a Foundation Scholarship, and proceeded to the B A degree in 1869, being 26th Wrangler in the Mathematical Tripos. After taking his degree he studied for a medical career, and in the course of his preliminary scientific work he attended the lectures on Elementary Biology and Physiology given by Michael Foster, who came to Cambridge as Prælector in Physiology at Trinity College in 1870 Foster led a considerable number of his early pupils to a scientific career He first aroused an interest in scientific problems and then, sometimes gradually, sometimes suddenly, suggested that there was no better course in life than that of trying to solve them Gaskell, as far as my recollection serves, was influenced in the latter way In 1872 he went to University College Hospital, London, for clinical work On his return to Cambridge, Foster, in the course of a conversation with him, suggested he should drop his medical career for the time and try his hand at research in physiology. Gaskell (I believe) adopted on the spot this suggestion, and instead of proceeding to the M B degree went to Leipzig to work under Ludwig (1874)

At this time Ludwig's laboratory was much the most important school of physiological research in Germany or elsewhere It attracted students from all parts of the world. All the work was planned by Ludwig, who had an almost unerring sense of the lines of work which would yield profitable results. To this the success of the school was mainly due. Its popularity was increased by the method of procedure adopted by Ludwig. This has been described by Sir T Lauder Brunton, who was with Ludwig in 1869–70 The experiments were carried out by Ludwig with the pupil as assistant, Ludwig wrote the paper and then published it, occasionally as a conjoint work, but more usually in the name of his pupil. As I have heard from Gaskell, the method was the same in his time. The work given him was a continuation of that on the innervation of skeletal muscle already

begun in the laboratory. This led him by a series of steps, which were perfectly logical, but impossible to foresee, from point to point of scientific enquiry up to his theory of the origin of vertebrates.

Soon after his return to England in 1875, Gaskell married Miss Catherine Sharpe Parker, a daughter of Mr R A Parker, of the firm of Messrs. Sharpe, Parker, and Co, solicitors, by whom he had one son, Dr. J. F Gaskell, and four daughters, two of whom survive him He settled in Grantchester, about a mile and a half from Cambridge, and in the Cambridge Physiological Laboratory he carried further the investigation on the innervation of the blood vessels of striated muscle He found (1877), amongst other facts, that stimulation of the nerve supplying the mylohyoid muscle of the frog caused considerable and constant dilatation of the blood vessels, although contraction of the muscle itself was prevented by curare This was the most decisive instance known at the time of such action in a purely muscular structure It did not, however, settle the question of the occurrence of vaso-dilator fibres in the nerves of skeletal muscle, the discussion of which was carried on by Heidenhain and others

From the behaviour of the arteries under nervous stimulation he passed to the investigation of the behaviour of the small arteries and of the heart with varying reaction of the blood, and, finding that a small addition of alkali increased the tone of both, and that a small addition of acid decreased it, he suggested that, besides the nervous control of the circulation, there was also a chemical control in each organ and tissue by the products set free in activity, so that, for example, the contraction of the muscle by setting free acid led to an increased flow of blood through it The suggestion was not entirely new, but it was wider in range than any of its kind previously made and rested on more solid facts. This work directed his attention to the heart, and for the next four or five years he devoted his time to the questions of the innervation of the heart, and the cause of the heart beat. With these questions others were busily engaged, notably Engelmann and Heidenhain.

In the early seventies it was universally held that the beat of the heart was due to the nerve cells present in it, and that it was initiated by the nerve cells of the sinus venosus There were very varied views as to the method of working of the nervous mechanism, especially as to the parts played by the nerve cells of the septum of the auricle, and the nerve cells of the base of the ventricle. As it became more widely recognised that parts of the heart which had no discernible nerve cells could contract rhythmically, it was felt that the nervous theory did not account for the whole of the phenomena. Moreover, some of the pharmacological results could not be satisfactorily explained on the theory as then put forward. But no one had any more satisfactory explanation to offer.

The question of the action of the nerve cells in the heart was part of the general question of the functions of the peripheral ganglia. In 1869, Engelmann argued that the peristaltic contraction of the ureters did not depend on nerve cells and that the contraction was conducted from one

muscle cell to the next without the intervention of nerve fibres. In 1875 he advocated a similar view as regards the passage of contraction from one part of the ventricle of the frog's heart to the rest, and he thought this was probably also the case in the auricle But in one important point he kept to the old theory and considered that the passage of contraction from auricle to ventricle was brought about by nerve cells and nerve fibres Gaskell (1881) at first adopted the current theory with some modifications in detail, but in 1883 he abandoned it, and argued that the contraction of the heart was of muscular origin, it started in the sinus and spread as a peristaltic wave to the other chambers, the delay in the passage of the contraction wave from one chamber of the heart to the next being due to a slow conduction in the modified muscular tissue which he found at the junction of the sinus venosus with the auricle, and at the junction of the auricle with the ventricle In the course of his work Gaskell made a large number of original observations on the behaviour of the several parts of the heart and of the cardiac muscle The term "block" Gaskell adopted from Romanes' account of the passage of contraction waves in Medusæ, the phenomena had been partly worked out in the frog's ventricle by Engelmann, but they were much more completely elucidated by Gaskell's work on the heart of the frog and the tortoise It was known that the contraction of the ventricle might only occur at every second, third, or fourth beat of the auricle. Gaskell obtained this effect experimentally by varying the degree of block between the two chambers After the lapse of years the invention of the string galvanometer brought the observation of heart block in man into the region of clinical medicine

The different effects produced on the heart of the frog by stimulating the vagus nerve were investigated simultaneously by Gaskell and by Heidenhain Gaskell observed that stimulation of the vagus sometimes caused an increase in the strength of the beats in addition to the quickening which had been already described by Schmiedeberg and others, and which had been attributed to special accelerator nerve fibres. Heidenhain found that by stimulating the medulla oblongata at different points, acceleration and augmentation, or slowing and weakening, of the heart beat could be obtained Gaskell traced in the crocodile and frog the origin of the accelerator fibres to the sympathetic system, and this was followed up by a more complete anatomical investigation by Gaskell and Gadow The innervation of the heart of lower vertebrates was thus brought into line with that of the mammal. In addition, he gave a more complete account than had been given by Heidenhain of the cause of the independence of the slowing and the weakening of the heart beat caused by pure vagus fibres, and of the quickening and the increase of strength caused by sympathetic fibres A little later Gaskell showed that an electrical change can be produced in quiescent heart muscle on stimulation of the cardiac nerves, and that the change is different according as the vagus or the accelerator nerve is stimulated.

Gaskell's work in this field was of the first importance His papers are a storehouse of observations of a fundamental nature He elaborated his theories and gave an admirable account of the whole subject in an article on "The Contraction of Cardiac Muscle" in Schafer's 'Text Book of Physiology,' published in 1900 It may be mentioned that the rhythm of the heart was the subject of his Croonian Lecture to the Royal Society in 1881, and that on the work mentioned above he was elected a Fellow of the Society in the following year

In the course of his dissection of the accelerator nerve in mammals, Gaskell was struck by the overwhelming preponderance of non-medullated nerve fibres in it, although the nerves centrally of ganglia from which the accelerator fibres arose were mainly medullated, and this determined him to investigate the relation of the sympathetic system to the spinal cord. At this time the question of the relation of the sympathetic and other peripheral ganglia to the cerebro-spinal system was in a state of profound confusion, and general agreement had been reached on a few points only A great number of facts had been described, and they covered a wide area of descriptive anatomy in different classes of vertebrates, of histology of nerve fibres and nerve cells, and of physiology Few observers covered more than a small portion of the ground Results were coming quickly and the ground was tilled rather hastily The practical disappearance of the theory that the "vegetative" nervous system was independent of the "animal" nervous system had led to the peripheral ganglia being less considered as a whole than they had been at an earlier time, and to special explanations being put forward for the working of the several parts. Thus, those writers who tried to give an impartial summary of the state of knowledge found themselves reduced to stating a number of more or less contradictory facts and irreconcilable theories

Gaskell did not approach the subject from the point of view of what had already been done or said He approached it from the point of view suggested by his observations on the accelerator nerves in the mammal This method had the disadvantage that it led him to leave uninvestigated some of the chief difficulties which were felt at the time, but it had the advantage that it enabled him to come to a rapid decision on certain important points Gaskell confined his attention to the efferent "visceral" fibres. His most important conclusions were, that all efferent visceral fibres, whether in cranial or in spinal nerves, were small medullated fibres, and that they left the cerebro-spinal system in three groups—the cervico-cranial, the thoracic, and the sacral—the thoracic portion being what was ordinarily called the sympathetic. These conclusions re-established the connection of small medullated fibres with the whole of the "organic" system described by Bidder and Volkmann in 1842, gave an explanation of Reissner's statement in 1862 that the anterior roots of the thoracic nerves contained bundles of small medullated fibres, whilst those of the cervical and lumbar nerves contained only a few such fibres scattered amongst the larger ones, supported the view which had been

held by some anatomists that the white rami communicantes constituted the sole connection between the spinal cord and the sympathetic, and brought all the involuntary nerves of whatever origin into one system of ganglionated nerves as had been recently advocated by Dastre and Morat.

In these conclusions there was one weak spot. Whilst it was definitely shown that the outflow of visceral fibres from the central nervous system to the sympathetic was enormously greater in the regions in which there were only white rami, it was not shown that no fibres passed out by the grey rami. Gaskell's observation of the rarity of small medullated fibres in the grey rami was not in accord with earlier observations, and he did in fact under-estimate their number. Further, physiologists of repute had described vaso-motor, pupil or heart effects as being caused by stimulation of the cervical nerves, which had grey rami only. It might then be said that the few small medullated fibres present in the centrally running branch of the grey rami represented the few scattered small medullated fibres of the anterior roots of the corresponding spinal nerves. Thus the difference between the thoracic and other regions of the spinal cord might be one of degree only. So far, however, as subsequent investigation has gone, Gaskell's conclusion was correct, and the grey rami receive no efferent fibres from the spinal cord. Gaskell's work clarified the air. It gave anatomists and physiologists a clearer view of the general arrangement of the efferent nerves governing unstriated muscle and glands, and it directed the attention of physiologists to points which they had singularly neglected. It is to be noticed also that Gaskell's earlier theory that the heart-beat is not due to the activity of local nerve cells has an intimate bearing on the much discussed question of the automatic and reflex action of peripheral ganglia.

In the paper setting forth the conclusions given above, Gaskell discussed a number of other problems of the sympathetic system. His theories were based on facts known at the time, but the experiments to test their wider application were few. Some are still under discussion, some are superseded. The most far-reaching of these theories was on the nature of the difference between motor and inhibitory nerve fibres. In 1881 he had advocated the view that the vagus is the trophic nerve of the heart. Löwit, in 1882, had suggested, on the lines of Hering's theory of assimilatory and dissimilary processes in the body, that the cardiac inhibitory fibres favour assimilation, and that the accelerator fibres favour dissimilation. Gaskell, developing his trophic theory, took a more definite and a wider view and urged that all inhibitory fibres are anabolic, and all motor fibres are katabolic.

Gaskell's microscopical and anatomical observations led him to questions of morphology. He argued that in a typical spinal segment a lateral root was to be distinguished in addition to the ventral and dorsal roots. The lateral root consisted of two parts, one arose from the lateral mesoblast plates of van Wijhe and supplied the respiratory muscles of Ch. Bell's system, the other formed the ganglionated nerves of the visceral system. On this basis

he discussed the homologies of the cranial and spinal nerves, and returned to this subject in a paper published a few years later. For his work on the nervous system he was awarded the Marshall Hall Prize of the Royal Medical and Chirurgical Society in 1888, and was elected an Honorary Fellow of the Society.

In 1890, the Nizam of Hyderabad supplied funds to a Commission for the investigation of the cause of death under chloroform—the second which he had supported The Commission reported that death was due to an action of the respiratory centre, and that if the respiration were carefully attended to it was unnecessary to pay any attention to the pulse These conclusions were directly opposed to common belief based both on experimental and clinical observation One of the members of the Commission asked Gaskell to criticise their Report Gaskell arranged with Dr Shore to make a joint experimental enquiry Gaskell and Shore, employing various methods, notably that of cross circulation from one animal to another, brought forward evidence, which was generally regarded as conclusive, that chloroform had a direct weakening action on the heart Their paper, published in 1893, checked a tendency to regard the respiration as the only factor to be considered in administering chloroform It was a useful piece of work, but it gave Gaskell the only enemy he ever made

This investigation was a side track from the main line of the work which Gaskell had been pursuing for some years His morphological studies on the homologies of the cranial and spinal nerves had led him to consider the problem of the origin of the nervous system in vertebrates, and this again led him to a theory of the origin of vertebrates to which he gave nearly all his time in later years. Dr. Gadow has been kind enough to write the following account of this part of Gaskell's researches —

"Gaskell's physiological research has always been to a considerable extent on the morphological side, and this combination of the sister sciences culminated in his enquiry into the origin of vertebrates He was drawn to this at present hopelessly difficult problem neither by accident nor design but by the complete failure of various morphological friends to account for certain structures the understanding of which was necessary for his research. He therefore determined to find out for himself, and thus it has come to pass that a man between 30 and 40 years of age, M D. of Cambridge and a physiologist of renown, devoted about 25 years of his life to essentially morphological studies, more than—in the nature of things—applies to some of his rather bitter scientific opponents Moreover, entering the new field quite unbiassed, his critical mind enabled him, when studying for instance the best comprehensive text-books on embryology, to discover the weak sides of that discipline It was not a question of picking out what suited him, on the contrary there was hardly a point—be it the homologies of the germinal layers, the occurrence of some obscure feature like Reissner's fibre, or some Silurian fossil, which he did not take often infinite pains to examine into. Frequently he enlisted friendly help, as in the case of the digestive properties of the lamprey's skin.

"This is not the place to discuss the strong and weak points of his hypothesis that vertebrates are descended from some Crustacean-like ancestor, *i.e.* from some vaguely reconstructable stock of which the palæozoic Trilobites, King crabs and Scorpions are the only known representatives on the invertebrate side, and he bridged the gulf between them and the vertebrates by the Silurian Ostracoderms, of whose internal organisation the larvæ of the Lampreys, before their marvellous changes into the present adult forms, seemed to afford a clue. The gulf was great indeed, but his planned bridges were not more hazily sketched than those which pretend to connect the vertebrates either separately or conjointly with Amphioxus, Tunicates, Balanoglossus, etc., with worms and even with Echinoderms. Especially the various worm-theories he considered as no solution of the problem, since they would carry the connection so far back as to merge almost into the beginning of the Metazoa, amounting to no recognisable origin. He on the contrary believed that 'each higher group of animals has arisen in succession from the highest race developed up to that time.'

"Further, as the leading motif of the whole course of this solution he discerned the orderly sequence in the development of the central nervous system, in which no break of continuity can possibly have occurred. The brain and nerves afford the fundamental homologies; the organs which they innervate may fall into line in a surprising way, but they are not the essential comparisons, *e.g.* a new gut may be formed, as in the transforming Ammocœtes. 'The secret of evolutionary success is the development of a superior brain.'

"The immediate starting point of Gaskell's investigations on the origin of vertebrates was the recognition of the close similarity in structure and function of the different parts of the vertebrate brain with those of Arthropods. The segmental character of the vertebrate central nervous system, so clear to the physiologist, and long before insisted upon by most anatomists, had lost weight for the morphologists, clearly because the C.N.S. appears embryonically as a single unsegmented tube. Here then was the next question forced upon Gaskell's attention. Cannot the two opposing views be reconciled by the suggestion that the vertebrate C.N.S. consists of two parts, closely entangled, viz., a segmental nervous system on the same plan as that of the Arthropods, which is outside and has surrounded an epithelial tubular structure?

"This idea explained at once the remarkable non-nervous epithelial parts of the tube, which become so conspicuous as we descend the vertebrate phylum, and every part of this tube bears the same resemblance to various parts of the C.N.S. as the dorsal stomach and intestine of an Arthropod. As a crowning of his conception the pineal eyes fit into the right place of the scheme; and the resemblances become greater and more numerous on the one hand in Ammocœtes, as was to be expected in the lowest available vertebrate, and on the other in Limulus, the King crab. In short, there was now a provisional working hypothesis, obtained by a direct logical process from the consideration of the vertebrate nervous system.

"After this working explanation of the tubular nature of the C.N.S the next step was the enquiry into the nature of the cranial nerves and, therefore, the double segmentation of the vertebrate body in the head region Now he was in the midst of the most complex and abstruse problem of morphology, involving every organic system The resemblances between Arthropods and vertebrates—with Limulus and Ammocœtes as the champions—are indeed numerous and in many cases perplexingly close Of course, the more Gaskell became absorbed by his research, the more resemblances he saw, many of which are in all probability mere coincidences, or even erroneous With great intuition and ingenuity he connected them, and in some of the most important cases his argumentation as to their being homologous structures has remained intact. He knew that if but a few are true homologies, his case would be proven, according to all the accepted canons of the theory of descent, and all the rest could be waived aside as incidental convergences, due to correlations, the possible laws of which we are now only just beginning to speculate about. Hence he felt it necessary to defend, so to speak, his whole extended line, not that the yielding of some point would mean a disastrous breach, but because of the lack of criterion to know which of his many points might prove one of his best assets, viz, an absolute homologue

"On the other hand he felt justified in assuming as most unlikely that representatives of two fundamentally different phyla should have produced so very many close resemblances, so close in function, structure, and relative position as to make it impossible to show them up as heterogenous He was also fully aware of it that our time-honoured conception of homologies versus analogies and their application to phylogeny are under reconsideration It is a blow to the comparative anatomist and to the constructor of pedigrees, but all the more interesting since it shows that it is life, function, adaptation, and inheritance, which shape the material, and this being Gaskell's standpoint of view he skilfully worked with the tools of the morphologist as a physiologist. Be his genial hypothesis, elaborate enough for a theory, right or wrong, he has discovered and elucidated many a feature both in vertebrates and invertebrates which without his tireless work would remain still neglected and unexplained

"His book, "The Origin of Vertebrates," published in 1908, has made little impression. Partly it is to a great extent a reprint of numerous previous papers and series of essays, partly because, instead of pleading, he did not present his views and the long chain of argumentation in an easy manner Lastly the idea of our descent from 'some Crustacean-like ancestor' was so subversive of all the other rival hypotheses (one of which if assumed to be right implies that all the others are wrong) that the unbiassed reader expects at least a clearly summarising explanation why Gaskell considered the older hypotheses not only insufficient but wrong.

"He did not choose this line. He had too noble a character, the respecting admiration of his many friends, ever ready to defend his own, willing to give

in to sound argument, but not to be suppressed 'By their fruits you shall know them.'—H F. G"

In reviewing Gaskell's work one cannot fail to be struck with the carefulness and accuracy of his observations But the bent of his mind lay in the direction of generalisation. A fact once definitely ascertained was never viewed by him as an isolated phenomenon, it was used as a basis for formulating some general rule. If he sometimes generalised too hastily, it was but the defect of his virtue The value of his work was widely recognised. He was awarded a Royal Medal of the Royal Society in 1889, and at various times was the recipient of honours both at home and abroad

One or two further events of his life and some personal characteristics remain to be mentioned In 1878 he proceeded to the Degree of M D by Thesis, but he did not at any time practise medicine Two or three years after this he began a life-long part in the advanced teaching of Physiology in the University His subjects were those on which he had himself worked, viz, the heart, the nervous mechanism of respiration, the sympathetic system, and, at a later date, the origin of vertebrates In 1883 he was appointed University Lecturer His style was incisive, and he spoke on controversial points with a half-suppressed enthusiasm which was eminently infectious.

In 1888 he left Grantchester and took up his residence in Cambridge In the following year he was elected a Fellow of Trinity Hall, and was appointed Prælector in Natural Science in the College Living in a town was not to his liking, and in 1893 he built a house—The Uplands—on a hill-top in Great Shelford, opposite that on which perched Michael Foster's house. Here he remained for the rest of his life.

Gaskell attended but little the Congresses of Scientific Associations, though he did not altogether shun them He was President of Section I of the British Association in 1896 at Liverpool, and attended the meetings of the Association in Canada in 1897, and in South Africa in 1905, and took the opportunity of seeing a good deal of these countries. He was present also at one or two of the earlier triennial meetings of the International Congress of Physiologists. He did not take much interest in the ordinary business of the University, but he served on the University Council (1907–1910), and if any broad question came before the Senate he was fairly certain to be found on the Placet side When there was real need of his services he did not grudge them He served on the Royal Commission on Vivisection which was appointed in 1906, and the final report of which was not issued till 1912; and he was a member of the Mosely Commission on Education in America.

As an undergraduate he rowed in the May races, played cricket and racquets, and frequented the bathing sheds. Later on he enjoyed an occasional set of lawn tennis, but, in general, active exercise did not greatly attract him. In recreation, as, indeed, in work, he took throughout life a somewhat leisurely course. He liked both work and play,

but not to the stage of exhaustion. For some years he spent part of the Long Vacation yachting and fishing with his brother His hobby was gardening He converted a large part of his 15 acres of sloping hillside at Shelford into a charming terraced garden, the early summer display of which was the occasion of an annual reception to Cambridge residents. He was always glad to receive physiologists visiting Cambridge, and his bluff, hearty greeting left no doubt of their welcome In the evening he liked a game of whist or bridge, and after college feasts he was amongst the first to settle down to a rubber

In the year preceding his death he was a little troubled about his health, but his customary course of life was hardly affected He was writing a small volume on the 'Involuntary Nervous System,' and on September 3 revised the last sheets Early on the following morning he had cerebral hæmorrhage, and died on September 7 without recovering consciousness

J N L.

JOSEPH REYNOLDS GREEN, 1848-1914

JOSEPH REYNOLDS GREEN was born at Stowmarket, Suffolk, in 1848 He was destined for a commercial career, and actually entered upon it for some years But his real bent and capacity were scientific, and all his spare time was given to study, with the result that he took the B.Sc. degree of the University of London in 1880 This seems to have decided him to devote himself entirely to scientific work, and with this object in view he entered Trinity College, Cambridge, as a sizar, in October, 1881 He pursued his studies with such zeal and success that he was elected to a scholarship at Trinity in 1882, and gained a First Class in Part I of the Natural Sciences Tripos in 1883, as also a First Class in Part II the following year, his subjects being Botany and Animal Physiology. His work in the latter subject was carried on under the late Sir Michael Foster, whilst I was responsible for his botanical work He impressed us both, as a student, not only by his enthusiasm but also and more especially by the singular lucidity of his mind.

Having thus satisfactorily completed his undergraduate career, he at once applied himself to research, both botanical and physiological. His first published contribution to science was a paper on the glands of the Hypericaceæ, which appeared in the Journal of the Linnean Society, 1884. At the same time he was engaged in experiments upon the clotting of blood, which led him to make the important discovery that the process is dependent upon the presence of a calcium salt, more especially the sulphate, which, he concluded

affects either the formation or the activity of thrombin ("On Certain Points connected with the Coagulation of the Blood," 'Journ. Physiol,' 1887).

Green did not at once decide between the two sciences, which seemed to have equal attractions for him. His appointment in 1885 by Sir Michael Foster as Demonstrator in Animal Physiology, a position that he held with great credit for two years, determined the nature of his work for the time. But even so he continued to pursue more or less botanical research, the results of which were published in two papers read before the Royal Society, the one on the protein constituents of latex ('Proc Roy. Soc,' 1886), the other, of greater importance, on the changes in the proteins of the seed which accompany germination ('Phil Trans,' 1887) in which he confirmed for the Lupin the discovery by von Gorup-Besanez (1874) of a proteoclastic enzyme in the seed of the Vetch, and amplified it by showing that the protease is tryptic in its action. These papers indicated the direction of his future work.

In 1887 Green was appointed Professor of Botany to the Pharmaceutical Society of Great Britain, and consequently he devoted himself wholly to that science. During the 20 years that he held this office his literary output was voluminous. The first 12 volumes of the 'Annals of Botany' (1888–98) contain a number of papers by him on various points in the biochemistry of plants, and he contributed several articles to the first series (1894–8) of 'Science Progress.' The most important results of his investigations during this period were the following:—The discovery ('Ann Bot,' vol 1, 1888) that the conversion of inulin into sugar (fructose) during the germination of the Jerusalem artichoke is effected by a specific enzyme, inulase, the detection of a fat-splitting enzyme (lipase) in the germinating seed of the castor-oil plant ('Roy Soc Proc,' 1890), a subject to which he returned years afterwards ('Roy Soc Proc,' 1905), the demonstration of the presence and activity of amyloclastic enzymes in the germinating pollen-grain and in the tissue of the style ('Phil Trans,' 1894), the analysis of the action of light upon diastase ('Phil Trans,' 1897), showing that whereas the red, orange, and blue rays favour the formation of the enzyme, the green, the violet, and especially the ultra-violet rays destroy it, with the striking suggestion that "vegetable structures have a power of absorbing radiant energy which is not connected with the presence and activity of chlorophyll."

In his later years Green turned his attention mainly to the writing of books, and produced several considerable works, characterised by the lucidity of exposition that he possessed in a high degree. Three of them were text-books: 'A Manual of Botany based upon that of the late R. Bentley' (1895–6), 'An Introduction to Vegetable Physiology' (1900), and 'The Soluble Ferments and Fermentation' (1899). All three went to a second edition, but the third was the most important and successful of them; a German translation of it, by Windisch, was published in 1901. Further, he was commissioned by the delegates of the Clarendon Press, Oxford, to write a continuation of Sachs' 'History of Botany' (1530–1860), to bring the record up to the end

of the nineteenth century—a difficult task, which he performed with a considerable measure of success. He became so interested in work of this kind that he subsequently wrote a history of botany in England, which, unfortunately, has not yet been published.

Owing to failing health Green resigned his Professorship at the Pharmaceutical Society in 1907, and undertook the less onerous duties of the Hartley Lectureship on Vegetable Physiology in the University of Liverpool, a post that he held until his death His health finally broke down in September, 1913, when he had a stroke, from which he only partially recovered A second stroke, following an operation, carried him off on June 3, 1914, to the deep regret of his numerous friends, to whom he had endeared himself by the geniality of his disposition and his unfailing scientific enthusiasm

His merits did not pass unrecognised He proceeded M.A at Cambridge in 1888, D Sc in 1894; he became a Fellow of the Linnean Society in 1889, and was elected to the Royal Society in 1895. He was President of Section K (Botany) at the Belfast meeting of the British Association in 1902, and was elected, in the same year, Fellow of Downing College, Cambridge

S. H. V.

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